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(54) Title: RECEPTOR ACTIVATOR OF NF-KAPPA B, RECEPTOR IS MEMBER OF TNF RECEPTOR SUPERFAMILY			
(57) Abstract Isolated receptors, DNAs encoding such receptors, and pharmaceutical compositions made therefrom, are disclosed. The isolated receptors can be used to regulate an immune response. The receptors are also useful in screening for inhibitors thereof.			

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TITLE

RECEPTOR ACTIVATOR OF NF-KAPPA B, RECEPTOR IS MEMBER OF TNF RECEPTOR SUPERFAMILY

TECHNICAL FIELD OF THE INVENTION

The present invention relates generally to the field of cytokine receptors, and more specifically to cytokine receptor/ligand pairs having immunoregulatory activity.

BACKGROUND OF THE INVENTION

Efficient functioning of the immune system requires a fine balance between cell proliferation and differentiation and cell death, to ensure that the immune system is capable of reacting to foreign, but not self antigens. Integral to the process of regulating the immune and inflammatory response are various members of the Tumor Necrosis Factor (TNF) Receptor/Nerve Growth Factor Receptor superfamily (Smith et al., *Science* 248:1019; 1990). This family of receptors includes two different TNF receptors (Type I and Type II; Smith et al., *supra*; and Schall et al., *Cell* 61:361, 1990), nerve growth factor receptor (Johnson et al., *Cell* 47:545, 1986), B cell antigen CD40 (Stamenkovic et al., *EMBO J.* 8:1403, 1989), CD27 (Camerini et al., *J. Immunol.* 147:3165, 1991), CD30 (Durkop et al., *Cell* 68:421, 1992), T cell antigen OX40 (Mallett et al., *EMBO J.* 9:1063, 1990), human *Fas* antigen (Itoh et al., *Cell* 66:233, 1991), murine 4-1BB receptor (Kwon et al., *Proc. Natl. Acad. Sci. USA* 86:1963, 1989) and a receptor referred to as Apoptosis-Inducing Receptor (AIR; USSN 08/720,864, filed October 4, 1996).

CD40 is a receptor present on B lymphocytes, epithelial cells and some carcinoma cell lines that interacts with a ligand found on activated T cells, CD40L (USSN 08/249,189, filed May 24, 1994). The interaction of this ligand/receptor pair is essential for both the cellular and humoral immune response. Signal transduction via CD40 is mediated through the association of the cytoplasmic domain of this molecule with members of the TNF receptor-associated factors (TRAFs; Baker and Reddy, *Oncogene* 12:1, 1996). It has recently been found that mice that are defective in TRAF3 expression due to a targeted disruption in the gene encoding TRAF3 appear normal at birth but develop progressive hypoglycemia and depletion of peripheral white cells, and die by about ten days of age (Xu et al., *Immunity* 5:407, 1996). The immune responses of chimeric mice reconstituted with TRAF3^{-/-} fetal liver cells resemble those of CD40-deficient mice, although TRAF3^{-/-} B cells appear to be functionally normal.

The critical role of TRAF3 in signal transduction may be in its interaction with one of the other members of the TNF receptor superfamily, for example, CD30 or CD27, which are present on T cells. Alternatively, there may be other, as yet unidentified

members of this family of receptors that interact with TRAF3 and play an important role in postnatal development as well as in the development of a competent immune system. Identifying additional members of the TNF receptor superfamily would provide an additional means of regulating the immune and inflammatory response, as well as potentially providing further insight into post-natal development in mammals.

SUMMARY OF THE INVENTION

The present invention provides a novel receptor, referred to as RANK (for receptor activator of NF- κ B), that is a member of the TNF receptor superfamily. RANK is a Type I transmembrane protein having 616 amino acid residues that interacts with TRAF3. Triggering of RANK by over-expression, co-expression of RANK and membrane bound RANK ligand (RANKL), and with addition of soluble RANKL or agonistic antibodies to RANK results in the upregulation of the transcription factor NF- κ B, a ubiquitous transcription factor that is most extensively utilized in cells of the immune system.

Soluble forms of the receptor can be prepared and used to interfere with signal transduction through membrane-bound RANK, and hence upregulation of NF- κ B; accordingly, pharmaceutical compositions comprising soluble forms of the novel receptor are also provided. Inhibition of NF- κ B by RANK antagonists may be useful in ameliorating negative effects of an inflammatory response that result from triggering of RANK, for example in treating toxic shock or sepsis, graft-versus-host reactions, or acute inflammatory reactions. Soluble forms of the receptor will also be useful in vitro to screen for agonists or antagonists of RANK activity.

The cytoplasmic domain of RANK will be useful in developing assays for inhibitors of signal transduction, for example, for screening for molecules that inhibit interaction of RANK with TRAF2 or TRAF3. Deleted forms and fusion proteins comprising the novel receptor are also disclosed.

The present invention also identifies a counterstructure, or ligand, for RANK, referred to as RANKL. RANKL is a Type 2 transmembrane protein with an intracellular domain of less than about 50 amino acids, a transmembrane domain and an extracellular domain of from about 240 to 250 amino acids. Similar to other members of the TNF family to which it belongs, RANKL has a 'spacer' region between the transmembrane domain and the receptor binding domain that is not necessary for receptor binding. Accordingly, soluble forms of RANKL can comprise the entire extracellular domain or fragments thereof that include the receptor binding region.

These and other aspects of the present invention will become evident upon reference to the following detailed description of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 demonstrates the influence of RANK.Fc and hRANKL on activated T cell growth. Human peripheral blood T cells were cultured as described in Example 12; viable T cell recovery was determined by triplicate trypan blue countings.

Figure 2 illustrates the ability of RANKL to induce human DC cluster formation. Functionally mature dendritic cells (DC) were generated *in vitro* from CD34⁺ bone marrow (BM) progenitors and cultured as described in Example 13. CD1a⁺ DC were cultured in a cytokine cocktail alone (Figure 2A), in cocktail plus CD40L (Figure 2B), RANKL (Figure 2C), or heat inactivated (Δ H) RANKL (Figure 2D), and then photographed using an inversion microscope.

Figure 3 demonstrates that RANKL enhances DC allo-stimulatory capacity. Allogeneic T cells were incubated with varying numbers of irradiated DC cultured as described in Example 13. The cultures were pulsed with [³H]-thymidine and the cells harvested onto glass fiber sheets for counting. Values represent the mean \pm standard deviation (SD) of triplicate cultures.

Figure 4 presents an alignment of human RANK with other TNFR family members in the region of structurally conserved extracellular cysteine-rich pseudorepeats. Predicted disulfide linkages (DS1-DS3) are indicated. RANK and CD40 contain identical amino acid substitutions (C^H, C^G) eliminating DS2 in the second pseudorepeat.

Figure 5 presents an alignment of human RANKL with other TNF family members.

DETAILED DESCRIPTION OF THE INVENTION

A novel partial cDNA insert with a predicted open reading frame having some similarity to CD40 was identified in a database containing sequence information from cDNAs generated from human bone marrow-derived dendritic cells (DC). The insert was used to hybridize to colony blots generated from a DC cDNA library containing full-length cDNAs. Several colony hybridizations were performed, and two clones (SEQ ID NOs:1 and 3) were isolated. SEQ ID NO:5 shows the nucleotide and amino acid sequence of a predicted full-length protein based on alignment of the overlapping sequences of SEQ ID NOs:1 and 3.

RANK is a member of the TNF receptor superfamily; it most closely resembles CD40 in the extracellular region. Similar to CD40, RANK associates with TRAF2 and TRAF3 (as determined by co-immunoprecipitation assays substantially as described by Rothe et al., *Cell* 83:1243, 1995). TRAFs are critically important in the regulation of the immune and inflammatory response. Through their association with various members of the TNF receptor superfamily, a signal is transduced to a cell. That signal results in the proliferation, differentiation or apoptosis of the cell, depending on which receptor(s) is/are triggered and which TRAF(s) associate with the receptor(s); different signals can be

transduced to a cell via coordination of various signaling events. Thus, a signal transduced through one member of this family may be proliferative, differentiative or apoptotic, depending on other signals being transduced to the cell, and/or the state of differentiation of the cell. Such exquisite regulation of this proliferative/apoptotic pathway is necessary to develop and maintain protection against pathogens; imbalances can result in autoimmune disease.

RANK is expressed on epithelial cells, some B cell lines, and on activated T cells. However, its expression on activated T cells is late, about four days after activation. This time course of expression coincides with the expression of Fas, a known agent of apoptosis. RANK may act as an anti-apoptotic signal, rescuing cells that express RANK from apoptosis as CD40 is known to do. Alternatively, RANK may confirm an apoptotic signal under the appropriate circumstances, again similar to CD40. RANK and its ligand are likely to play an integral role in regulation of the immune and inflammatory response.

Moreover, the post-natal lethality of mice having a targeted disruption of the TRAF3 gene demonstrates the importance of this molecule not only in the immune response but in development. The isolation of RANK, as a protein that associates with TRAF3, and its ligand will allow further definition of this signaling pathway, and development of diagnostic and therapeutic modalities for use in the area of autoimmune and/or inflammatory disease.

DNAs, Proteins and Analogs

The present invention provides isolated RANK polypeptides and analogs (or muteins) thereof having an activity exhibited by the native molecule (i.e., RANK muteins that bind specifically to a RANK ligand expressed on cells or immobilized on a surface or to RANK-specific antibodies; soluble forms thereof that inhibit RANK ligand-induced signaling through RANK). Such proteins are substantially free of contaminating endogenous materials and, optionally, without associated native-pattern glycosylation. Derivatives of RANK within the scope of the invention also include various structural forms of the primary proteins which retain biological activity. Due to the presence of ionizable amino and carboxyl groups, for example, a RANK protein may be in the form of acidic or basic salts, or may be in neutral form. Individual amino acid residues may also be modified by oxidation or reduction. The primary amino acid structure may be modified by forming covalent or aggregative conjugates with other chemical moieties, such as glycosyl groups, lipids, phosphate, acetyl groups and the like, or by creating amino acid sequence mutants. Covalent derivatives are prepared by linking particular functional groups to amino acid side chains or at the N- or C-termini.

Derivatives of RANK may also be obtained by the action of cross-linking agents, such as M-maleimidobenzoyl succinimide ester and N-hydroxysuccinimide, at cysteine and

lysine residues. The inventive proteins may also be covalently bound through reactive side groups to various insoluble substrates, such as cyanogen bromide-activated, bisoxirane-activated, carbonyldiimidazole-activated or tosyl-activated agarose structures, or by adsorbing to polyolefin surfaces (with or without glutaraldehyde cross-linking). Once bound to a substrate, the proteins may be used to selectively bind (for purposes of assay or purification) antibodies raised against the proteins or against other proteins which are similar to RANK or RANKL, as well as other proteins that bind RANK or RANKL or homologs thereof.

Soluble forms of RANK are also within the scope of the invention. The nucleotide and predicted amino acid sequence of the RANK is shown in SEQ ID NOs:1 through 6. Computer analysis indicated that the protein has an N-terminal signal peptide; the predicted cleavage site follows residue 24. Those skilled in the art will recognize that the actual cleavage site may be different than that predicted by computer analysis. Thus, the N-terminal amino acid of the cleaved peptide is expected to be within about five amino acids on either side of the predicted, preferred cleavage site following residue 24. Moreover a soluble form beginning with amino acid 33 was prepared; this soluble form bound RANKL. The signal peptide is predicted to be followed by a 188 amino acid extracellular domain, a 21 amino acid transmembrane domain, and a 383 amino acid cytoplasmic tail.

Soluble RANK comprises the signal peptide and the extracellular domain (residues 1 to 213 of SEQ ID NO:6) or a fragment thereof. Alternatively, a different signal peptide can be substituted for the native leader, beginning with residue 1 and continuing through a residue selected from the group consisting of amino acids 24 through 33 (inclusive) of SEQ ID NO:6. Moreover, fragments of the extracellular domain will also provide soluble forms of RANK. Fragments can be prepared using known techniques to isolate a desired portion of the extracellular region, and can be prepared, for example, by comparing the extracellular region with those of other members of the TNFR family and selecting forms similar to those prepared for other family members. Alternatively, unique restriction sites or PCR techniques that are known in the art can be used to prepare numerous truncated forms which can be expressed and analyzed for activity.

Fragments can be prepared using known techniques to isolate a desired portion of the extracellular region, and can be prepared, for example, by comparing the extracellular region with those of other members of the TNFR family (of which RANK is a member) and selecting forms similar to those prepared for other family members. Alternatively, unique restriction sites or PCR techniques that are known in the art can be used to prepare numerous truncated forms which can be expressed and analyzed for activity.

Other derivatives of the RANK proteins within the scope of this invention include covalent or aggregative conjugates of the proteins or their fragments with other proteins or polypeptides, such as by synthesis in recombinant culture as N-terminal or C-terminal

fusions. For example, the conjugated peptide may be a signal (or leader) polypeptide sequence at the N-terminal region of the protein which co-translationally or post-translationally directs transfer of the protein from its site of synthesis to its site of function inside or outside of the cell membrane or wall (e.g., the yeast α -factor leader).

5 Protein fusions can comprise peptides added to facilitate purification or identification of RANK proteins and homologs (e.g., poly-His). The amino acid sequence of the inventive proteins can also be linked to an identification peptide such as that described by Hopp et al., *Bio/Technology* 6:1204 (1988). Such a highly antigenic peptide provides an epitope reversibly bound by a specific monoclonal antibody, enabling rapid
10 assay and facile purification of expressed recombinant protein. The sequence of Hopp et al. is also specifically cleaved by bovine mucosal enterokinase, allowing removal of the peptide from the purified protein. Fusion proteins capped with such peptides may also be resistant to intracellular degradation in *E. coli*.

Fusion proteins further comprise the amino acid sequence of a RANK linked to an
15 immunoglobulin Fc region. An exemplary Fc region is a human IgG₁ having a nucleotide an amino acid sequence set forth in SEQ ID NO:8. Fragments of an Fc region may also be used, as can Fc muteins. For example, certain residues within the hinge region of an Fc region are critical for high affinity binding to Fc γ RI. Canfield and Morrison (*J. Exp. Med.* 173:1483; 1991) reported that Leu₍₂₃₄₎ and Leu₍₂₃₅₎ were critical to high affinity binding of
20 IgG₃ to Fc γ RI present on U937 cells. Similar results were obtained by Lund et al. (*J. Immunol.* 147:2657, 1991; *Molecular Immunol.* 29:53, 1991). Such mutations, alone or in combination, can be made in an IgG₁ Fc region to decrease the affinity of IgG₁ for FcR. Depending on the portion of the Fc region used, a fusion protein may be expressed as a dimer, through formation of interchain disulfide bonds. If the fusion proteins are made
25 with both heavy and light chains of an antibody, it is possible to form a protein oligomer with as many as four RANK regions.

In another embodiment, RANK proteins further comprise an oligomerizing peptide such as a leucine zipper domain. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al., *Science* 240:1759, 1988). Leucine zipper
30 domain is a term used to refer to a conserved peptide domain present in these (and other) proteins, which is responsible for dimerization of the proteins. The leucine zipper domain (also referred to herein as an oligomerizing, or oligomer-forming, domain) comprises a repetitive heptad repeat, with four or five leucine residues interspersed with other amino acids. Examples of leucine zipper domains are those found in the yeast transcription factor
35 GCN4 and a heat-stable DNA-binding protein found in rat liver (C/EBP; Landschulz et al., *Science* 243:1681, 1989). Two nuclear transforming proteins, *fos* and *jun*, also exhibit leucine zipper domains, as does the gene product of the murine proto-oncogene, *c-myc* (Landschulz et al., *Science* 240:1759, 1988). The products of the nuclear oncogenes *fos*

and *jun* comprise leucine zipper domains preferentially form a heterodimer (O'Shea et al., *Science* 245:646, 1989; Turner and Tjian, *Science* 243:1689, 1989). The leucine zipper domain is necessary for biological activity (DNA binding) in these proteins.

The fusogenic proteins of several different viruses, including paramyxovirus, coronavirus, measles virus and many retroviruses, also possess leucine zipper domains (Buckland and Wild, *Nature* 338:547, 1989; Britton, *Nature* 353:394, 1991; Delwart and Mosialos, *AIDS Research and Human Retroviruses* 6:703, 1990). The leucine zipper domains in these fusogenic viral proteins are near the transmembrane region of the proteins; it has been suggested that the leucine zipper domains could contribute to the oligomeric structure of the fusogenic proteins. Oligomerization of fusogenic viral proteins is involved in fusion pore formation (Spruce et al, *Proc. Natl. Acad. Sci. U.S.A.* 88:3523, 1991). Leucine zipper domains have also been recently reported to play a role in oligomerization of heat-shock transcription factors (Rabindran et al., *Science* 259:230, 1993).

Leucine zipper domains fold as short, parallel coiled coils. (O'Shea et al., *Science* 254:539; 1991) The general architecture of the parallel coiled coil has been well characterized, with a "knobs-into-holes" packing as proposed by Crick in 1953 (*Acta Crystallogr.* 6:689). The dimer formed by a leucine zipper domain is stabilized by the heptad repeat, designated $(abcdefg)_n$ according to the notation of McLachlan and Stewart (*J. Mol. Biol.* 98:293; 1975), in which residues *a* and *d* are generally hydrophobic residues, with *d* being a leucine, which line up on the same face of a helix. Oppositely-charged residues commonly occur at positions *g* and *e*. Thus, in a parallel coiled coil formed from two helical leucine zipper domains, the "knobs" formed by the hydrophobic side chains of the first helix are packed into the "holes" formed between the side chains of the second helix.

The leucine residues at position *d* contribute large hydrophobic stabilization energies, and are important for dimer formation (Krystek et al., *Int. J. Peptide Res.* 38:229, 1991). Lovejoy et al. recently reported the synthesis of a triple-stranded α -helical bundle in which the helices run up-up-down (*Science* 259:1288, 1993). Their studies confirmed that hydrophobic stabilization energy provides the main driving force for the formation of coiled coils from helical monomers. These studies also indicate that electrostatic interactions contribute to the stoichiometry and geometry of coiled coils.

Several studies have indicated that conservative amino acids may be substituted for individual leucine residues with minimal decrease in the ability to dimerize; multiple changes, however, usually result in loss of this ability (Landschulz et al., *Science* 243:1681, 1989; Turner and Tjian, *Science* 243:1689, 1989; Hu et al., *Science* 250:1400, 1990). van Heekeren et al. reported that a number of different amino residues can be substituted for the leucine residues in the leucine zipper domain of GCN4, and further found that some GCN4 proteins containing two leucine substitutions were weakly active

(*Nucl. Acids Res.* 20:3721, 1992). Mutation of the first and second heptadic leucines of the leucine zipper domain of the measles virus fusion protein (MVF) did not affect syncytium formation (a measure of virally-induced cell fusion); however, mutation of all four leucine residues prevented fusion completely (Buckland et al., *J. Gen. Virol.* 73:1703, 5 1992). None of the mutations affected the ability of MVF to form a tetramer.

Amino acid substitutions in the *a* and *d* residues of a synthetic peptide representing the GCN4 leucine zipper domain have been found to change the oligomerization properties of the leucine zipper domain (Alber, Sixth Symposium of the Protein Society, San Diego, CA). When all residues at position *a* are changed to isoleucine, the leucine zipper still 10 forms a parallel dimer. When, in addition to this change, all leucine residues at position *d* are also changed to isoleucine, the resultant peptide spontaneously forms a trimeric parallel coiled coil in solution. Substituting all amino acids at position *d* with isoleucine and at position *a* with leucine results in a peptide that tetramerizes. Peptides containing these substitutions are still referred to as leucine zipper domains.

Also included within the scope of the invention are fragments or derivatives of the intracellular domain of RANK. Such fragments are prepared by any of the herein-mentioned techniques, and include peptides that are identical to the cytoplasmic domain of RANK as shown in SEQ ID NO:5, or of murine RANK as shown in SEQ ID NO:15, and those that comprise a portion of the cytoplasmic region. All techniques used in preparing 20 soluble forms may also be used in preparing fragments or analogs of the cytoplasmic domain (i.e., RT-PCR techniques or use of selected restriction enzymes to prepare truncations). DNAs encoding all or a fragment of the intracytoplasmic domain will be useful in identifying other proteins that are associated with RANK signalling, for example using the immunoprecipitation techniques described herein, or another technique such as a 25 yeast two-hybrid system (Rothe et al., supra).

The present invention also includes RANK with or without associated native-pattern glycosylation. Proteins expressed in yeast or mammalian expression systems, e.g., COS-7 cells, may be similar or slightly different in molecular weight and glycosylation pattern than the native molecules, depending upon the expression system. Expression of DNAs 30 encoding the inventive proteins in bacteria such as *E. coli* provides non-glycosylated molecules. Functional mutant analogs of RANK protein having inactivated N-glycosylation sites can be produced by oligonucleotide synthesis and ligation or by site-specific mutagenesis techniques. These analog proteins can be produced in a homogeneous, reduced-carbohydrate form in good yield using yeast expression systems. 35 N-glycosylation sites in eukaryotic proteins are characterized by the amino acid triplet Asn-A₁-Z, where A₁ is any amino acid except Pro, and Z is Ser or Thr. In this sequence, asparagine provides a side chain amino group for covalent attachment of carbohydrate. Such a site can be eliminated by substituting another amino acid for Asn or for residue Z,

deleting Asn or Z, or inserting a non-Z amino acid between A₁ and Z, or an amino acid other than Asn between Asn and A₁.

RANK protein derivatives may also be obtained by mutations of the native RANK or subunits thereof. A RANK mutated protein, as referred to herein, is a polypeptide homologous to a native RANK protein, respectively, but which has an amino acid sequence different from the native protein because of one or a plurality of deletions, insertions or substitutions. The effect of any mutation made in a DNA encoding a mutated peptide may be easily determined by analyzing the ability of the mutated peptide to bind its counterstructure in a specific manner. Moreover, activity of RANK analogs, muteins or derivatives can be determined by any of the assays described herein (for example, inhibition of the ability of RANK to activate transcription).

Analogues of the inventive proteins may be constructed by, for example, making various substitutions of residues or sequences or deleting terminal or internal residues or sequences not needed for biological activity. For example, cysteine residues can be deleted or replaced with other amino acids to prevent formation of incorrect intramolecular disulfide bridges upon renaturation. Other approaches to mutagenesis involve modification of adjacent dibasic amino acid residues to enhance expression in yeast systems in which KEX2 protease activity is present.

When a deletion or insertion strategy is adopted, the potential effect of the deletion or insertion on biological activity should be considered. Subunits of the inventive proteins may be constructed by deleting terminal or internal residues or sequences. Soluble forms of RANK can be readily prepared and tested for their ability to inhibit RANK-induced NF- κ B activation. Polypeptides corresponding to the cytoplasmic regions, and fragments thereof (for example, a death domain) can be prepared by similar techniques. Additional guidance as to the types of mutations that can be made is provided by a comparison of the sequence of RANK to proteins that have similar structures, as well as by performing structural analysis of the inventive RANK proteins.

Generally, substitutions should be made conservatively; i.e., the most preferred substitute amino acids are those which do not affect the biological activity of RANK (i.e., ability of the inventive proteins to bind antibodies to the corresponding native protein in substantially equivalent a manner, the ability to bind the counterstructure in substantially the same manner as the native protein, the ability to transduce a RANK signal, or ability to induce NF- κ B activation upon overexpression in transient transfection systems, for example). Examples of conservative substitutions include substitution of amino acids outside of the binding domain(s) (either ligand/receptor or antibody binding areas for the extracellular domain, or regions that interact with other, intracellular proteins for the cytoplasmic domain), and substitution of amino acids that do not alter the secondary and/or

tertiary structure of the native protein. Additional examples include substituting one aliphatic residue for another, such as Ile, Val, Leu, or Ala for one another, or substitutions of one polar residue for another, such as between Lys and Arg; Glu and Asp; or Gln and Asn. Other such conservative substitutions, for example, substitutions of entire regions having similar hydrophobicity characteristics, are well known.

Mutations in nucleotide sequences constructed for expression of analog proteins or fragments thereof must, of course, preserve the reading frame phase of the coding sequences and preferably will not create complementary regions that could hybridize to produce secondary mRNA structures such as loops or hairpins which would adversely affect translation of the mRNA.

Not all mutations in the nucleotide sequence which encodes a RANK protein or fragments thereof will be expressed in the final product, for example, nucleotide substitutions may be made to enhance expression, primarily to avoid secondary structure loops in the transcribed mRNA (see EPA 75,444A, incorporated herein by reference), or to provide codons that are more readily translated by the selected host, e.g., the well-known *E. coli* preference codons for *E. coli* expression.

Although a mutation site may be predetermined, it is not necessary that the nature of the mutation *per se* be predetermined. For example, in order to select for optimum characteristics of mutants, random mutagenesis may be conducted and the expressed mutated proteins screened for the desired activity. Mutations can be introduced at particular loci by synthesizing oligonucleotides containing a mutant sequence, flanked by restriction sites enabling ligation to fragments of the native sequence. Following ligation, the resulting reconstructed sequence encodes an analog having the desired amino acid insertion, substitution, or deletion.

Alternatively, oligonucleotide-directed site-specific mutagenesis procedures can be employed to provide an altered gene having particular codons altered according to the substitution, deletion, or insertion required. Exemplary methods of making the alterations set forth above are disclosed by Walder et al. (*Gene* 42:133, 1986); Bauer et al. (*Gene* 37:73, 1985); Craik (*BioTechniques*, January 1985, 12-19); Smith et al. (*Genetic Engineering: Principles and Methods*, Plenum Press, 1981); and U.S. Patent NOs. 4,518,584 and 4,737,462 disclose suitable techniques, and are incorporated by reference herein.

Other embodiments of the inventive proteins include RANK polypeptides encoded by DNAs capable of hybridizing to the DNA of SEQ ID NO:6 under moderately stringent conditions (prewashing solution of 5 X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0) and hybridization conditions of 50°C, 5 X SSC, overnight) to the DNA sequences encoding RANK, or more preferably under stringent conditions (for example, hybridization in 6 X SSC at 63°C overnight; washing in 3 X SSC at 55°C), and other sequences which are

degenerate to those which encode the RANK. In one embodiment, RANK polypeptides are at least about 70% identical in amino acid sequence to the amino acid sequence of native RANK protein as set forth in SEQ ID NO:5. In a preferred embodiment, RANK polypeptides are at least about 80% identical in amino acid sequence to the native form of RANK; most preferred polypeptides are those that are at least about 90% identical to native RANK.

Percent identity may be determined using a computer program, for example, the GAP computer program described by Devereux et al. (*Nucl. Acids Res.* 12:387, 1984) and available from the University of Wisconsin Genetics Computer Group (UWGCG). For fragments derived from the RANK protein, the identity is calculated based on that portion of the RANK protein that is present in the fragment

The biological activity of RANK analogs or muteins can be determined by testing the ability of the analogs or muteins to inhibit activation of transcription, for example as described in the Examples herein. Alternatively, suitable assays, for example, an enzyme immunoassay or a dot blot, employing an antibody that binds native RANK, or a soluble form of RANKL, can be used to assess the activity of RANK analogs or muteins, as can assays that employ cells expressing RANKL. Suitable assays also include, for example, signal transduction assays and methods that evaluate the ability of the cytoplasmic region of RANK to associate with other intracellular proteins (i.e., TRAFs 2 and 3) involved in signal transduction will also be useful to assess the activity of RANK analogs or muteins. Such methods are well known in the art.

Fragments of the RANK nucleotide sequences are also useful. In one embodiment, such fragments comprise at least about 17 consecutive nucleotides, preferably at least about 25 nucleotides, more preferably at least 30 consecutive nucleotides, of the RANK DNA disclosed herein. DNA and RNA complements of such fragments are provided herein, along with both single-stranded and double-stranded forms of the RANK DNA of SEQ ID NO:5, and those encoding the aforementioned polypeptides. A fragment of RANK DNA generally comprises at least about 17 nucleotides, preferably from about 17 to about 30 nucleotides. Such nucleic acid fragments (for example, a probe corresponding to the extracellular domain of RANK) are used as a probe or as primers in a polymerase chain reaction (PCR).

The probes also find use in detecting the presence of RANK nucleic acids in *in vitro* assays and in such procedures as Northern and Southern blots. Cell types expressing RANK can be identified as well. Such procedures are well known, and the skilled artisan can choose a probe of suitable length, depending on the particular intended application. For PCR, 5' and 3' primers corresponding to the termini of a desired RANK DNA sequence are employed to amplify that sequence, using conventional techniques.

Other useful fragments of the RANK nucleic acids are antisense or sense oligonucleotides comprising a single-stranded nucleic acid sequence (either RNA or DNA) capable of binding to target RANK mRNA (sense) or RANK DNA (antisense) sequences. The ability to create an antisense or a sense oligonucleotide, based upon a cDNA sequence
5 for a given protein is described in, for example, Stein and Cohen, *Cancer Res.* 48:2659, 1988 and van der Krol et al., *BioTechniques* 6:958, 1988.

Uses of DNAs, Proteins and Analogs

The RANK DNAs, proteins and analogs described herein will have numerous uses,
10 including the preparation of pharmaceutical compositions. For example, soluble forms of RANK will be useful as antagonists of RANK-mediated NF- κ B activation, as well as to inhibit transduction of a signal via RANK. RANK compositions (both protein and DNAs) will also be useful in development of both agonistic and antagonistic antibodies to RANK. The inventive DNAs are useful for the expression of recombinant proteins, and as probes
15 for analysis (either quantitative or qualitative) of the presence or distribution of RANK transcripts.

The inventive proteins will also be useful in preparing kits that are used to detect soluble RANK or RANKL, or monitor RANK-related activity, for example, in patient specimens. RANK proteins will also find uses in monitoring RANK-related activity in
20 other samples or compositions, as is necessary when screening for antagonists or mimetics of this activity (for example, peptides or small molecules that inhibit or mimic, respectively, the interaction). A variety of assay formats are useful in such kits, including (but not limited to) ELISA, dot blot, solid phase binding assays (such as those using a biosensor), rapid format assays and bioassays.

The purified RANK according to the invention will facilitate the discovery of inhibitors of RANK, and thus, inhibitors of an inflammatory response (via inhibition of NF- κ B activation). The use of a purified RANK polypeptide in the screening for potential inhibitors is important and can virtually eliminate the possibility of interfering reactions with contaminants. Such a screening assay can utilize either the extracellular domain of RANK,
30 the intracellular domain, or a fragment of either of these polypeptides. Detecting the inhibiting activity of a molecule would typically involve use of a soluble form of RANK derived from the extracellular domain in a screening assay to detect molecules capable of binding RANK and inhibiting binding of, for example, an agonistic antibody or RANKL, or using a polypeptide derived from the intracellular domain in an assay to detect inhibition
35 of the interaction of RANK and other, intracellular proteins involved in signal transduction.

Moreover, in vitro systems can be used to ascertain the ability of molecules to antagonize or agonize RANK activity. Included in such methods are uses of RANK chimeras, for example, a chimera of the RANK intracellular domain and an extracellular

domain derived from a protein having a known ligand. The effects on signal transduction of various molecule can then be monitored by utilizing the known ligand to transduce a signal.

In addition, RANK polypeptides can also be used for structure-based design of RANK-inhibitors. Such structure-based design is also known as "rational drug design." The RANK polypeptides can be three-dimensionally analyzed by, for example, X-ray crystallography, nuclear magnetic resonance or homology modeling, all of which are well-known methods. The use of RANK structural information in molecular modeling software systems to assist in inhibitor design is also encompassed by the invention. Such computer-assisted modeling and drug design may utilize information such as chemical conformational analysis, electrostatic potential of the molecules, protein folding, etc. A particular method of the invention comprises analyzing the three dimensional structure of RANK for likely binding sites of substrates, synthesizing a new molecule that incorporates a predictive reactive site, and assaying the new molecule as described above.

Expression of Recombinant RANK

The proteins of the present invention are preferably produced by recombinant DNA methods by inserting a DNA sequence encoding RANK protein or an analog thereof into a recombinant expression vector and expressing the DNA sequence in a recombinant expression system under conditions promoting expression. DNA sequences encoding the proteins provided by this invention can be assembled from cDNA fragments and short oligonucleotide linkers, or from a series of oligonucleotides, to provide a synthetic gene which is capable of being inserted in a recombinant expression vector and expressed in a recombinant transcriptional unit.

Recombinant expression vectors include synthetic or cDNA-derived DNA fragments encoding RANK, or homologs, muteins or bioequivalent analogs thereof, operably linked to suitable transcriptional or translational regulatory elements derived from mammalian, microbial, viral or insect genes. Such regulatory elements include a transcriptional promoter, an optional operator sequence to control transcription, a sequence encoding suitable mRNA ribosomal binding sites, and sequences which control the termination of transcription and translation, as described in detail below. The ability to replicate in a host, usually conferred by an origin of replication, and a selection gene to facilitate recognition of transformants may additionally be incorporated.

DNA regions are operably linked when they are functionally related to each other. For example, DNA for a signal peptide (secretory leader) is operably linked to DNA for a polypeptide if it is expressed as a precursor which participates in the secretion of the polypeptide; a promoter is operably linked to a coding sequence if it controls the transcription of the sequence; or a ribosome binding site is operably linked to a coding

sequence if it is positioned so as to permit translation. Generally, operably linked means contiguous and, in the case of secretory leaders, contiguous and in reading frame. DNA sequences encoding RANK, or homologs or analogs thereof which are to be expressed in a microorganism will preferably contain no introns that could prematurely terminate transcription of DNA into mRNA.

Useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and pGEM1 (Promega Biotec, Madison, WI, USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed. *E. coli* is typically transformed using derivatives of pBR322, a plasmid derived from an *E. coli* species (Bolivar et al., *Gene* 2:95, 1977). pBR322 contains genes for ampicillin and tetracycline resistance and thus provides simple means for identifying transformed cells.

Promoters commonly used in recombinant microbial expression vectors include the β -lactamase (penicillinase) and lactose promoter system (Chang et al., *Nature* 275:615, 1978; and Goeddel et al., *Nature* 281:544, 1979), the tryptophan (trp) promoter system (Goeddel et al., *Nucl. Acids Res.* 8:4057, 1980; and EPA 36,776) and tac promoter (Maniatis, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, p. 412, 1982). A particularly useful bacterial expression system employs the phage λ P_L promoter and cI857ts thermolabile repressor. Plasmid vectors available from the American Type Culture Collection which incorporate derivatives of the λ P_L promoter include plasmid pHUB2, resident in *E. coli* strain JMB9 (ATCC 37092) and pPLc28, resident in *E. coli* RR1 (ATCC 53082).

Suitable promoter sequences in yeast vectors include the promoters for metallothionein, 3-phosphoglycerate kinase (Hitzeman et al., *J. Biol. Chem.* 255:2073, 1980) or other glycolytic enzymes (Hess et al., *J. Adv. Enzyme Reg.* 7:149, 1968; and Holland et al., *Biochem.* 17:4900, 1978), such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase. Suitable vectors and promoters for use in yeast expression are further described in R. Hitzeman et al., EPA 73,657.

Preferred yeast vectors can be assembled using DNA sequences from pBR322 for selection and replication in *E. coli* (Amp^r gene and origin of replication) and yeast DNA sequences including a glucose-repressible ADH2 promoter and α -factor secretion leader. The ADH2 promoter has been described by Russell et al. (*J. Biol. Chem.* 258:2674, 1982) and Beier et al. (*Nature* 300:724, 1982). The yeast α -factor leader, which directs secretion

of heterologous proteins, can be inserted between the promoter and the structural gene to be expressed. See, e.g., Kurjan et al., *Cell* 30:933, 1982; and Bitter et al., *Proc. Natl. Acad. Sci. USA* 81:5330, 1984. The leader sequence may be modified to contain, near its 3' end, one or more useful restriction sites to facilitate fusion of the leader sequence to foreign genes.

The transcriptional and translational control sequences in expression vectors to be used in transforming vertebrate cells may be provided by viral sources. For example, commonly used promoters and enhancers are derived from Polyoma, Adenovirus 2, Simian Virus 40 (SV40), and human cytomegalovirus. DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early and late promoter, enhancer, splice, and polyadenylation sites may be used to provide the other genetic elements required for expression of a heterologous DNA sequence. The early and late promoters are particularly useful because both are obtained easily from the virus as a fragment which also contains the SV40 viral origin of replication (Fiers et al., *Nature* 273:113, 1978). Smaller or larger SV40 fragments may also be used, provided the approximately 250 bp sequence extending from the *Hind* III site toward the *Bgl* II site located in the viral origin of replication is included. Further, viral genomic promoter, control and/or signal sequences may be utilized, provided such control sequences are compatible with the host cell chosen. Exemplary vectors can be constructed as disclosed by Okayama and Berg (*Mol. Cell. Biol.* 3:280, 1983).

A useful system for stable high level expression of mammalian receptor cDNAs in C127 murine mammary epithelial cells can be constructed substantially as described by Cosman et al. (*Mol. Immunol.* 23:935, 1986). A preferred eukaryotic vector for expression of RANK DNA is referred to as pDC406 (McMahan et al., *EMBO J.* 10:2821, 1991), and includes regulatory sequences derived from SV40, human immunodeficiency virus (HIV), and Epstein-Barr virus (EBV). Other preferred vectors include pDC409 and pDC410, which are derived from pDC406. pDC410 was derived from pDC406 by substituting the EBV origin of replication with sequences encoding the SV40 large T antigen. pDC409 differs from pDC406 in that a *Bgl* II restriction site outside of the multiple cloning site has been deleted, making the *Bgl* II site within the multiple cloning site unique.

A useful cell line that allows for episomal replication of expression vectors, such as pDC406 and pDC409, which contain the EBV origin of replication, is CV-1/EBNA (ATCC CRL 10478). The CV-1/EBNA cell line was derived by transfection of the CV-1 cell line with a gene encoding Epstein-Barr virus nuclear antigen-1 (EBNA-1) and constitutively express EBNA-1 driven from human CMV immediate-early enhancer/promoter.

Host Cells

Transformed host cells are cells which have been transformed or transfected with expression vectors constructed using recombinant DNA techniques and which contain sequences encoding the proteins of the present invention. Transformed host cells may
5 express the desired protein (RANK, or homologs or analogs thereof), but host cells transformed for purposes of cloning or amplifying the inventive DNA do not need to express the protein. Expressed proteins will preferably be secreted into the culture supernatant, depending on the DNA selected, but may be deposited in the cell membrane.

Suitable host cells for expression of proteins include prokaryotes, yeast or higher
10 eukaryotic cells under the control of appropriate promoters. Prokaryotes include gram negative or gram positive organisms, for example *E. coli* or *Bacillus* spp. Higher eukaryotic cells include established cell lines of mammalian origin as described below. Cell-free translation systems could also be employed to produce proteins using RNAs derived from the DNA constructs disclosed herein. Appropriate cloning and expression
15 vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts are described by Pouwels et al. (*Cloning Vectors: A Laboratory Manual*, Elsevier, New York, 1985), the relevant disclosure of which is hereby incorporated by reference.

Prokaryotic expression hosts may be used for expression of RANK, or homologs or analogs thereof that do not require extensive proteolytic and disulfide processing.
20 Prokaryotic expression vectors generally comprise one or more phenotypic selectable markers, for example a gene encoding proteins conferring antibiotic resistance or supplying an autotrophic requirement, and an origin of replication recognized by the host to ensure amplification within the host. Suitable prokaryotic hosts for transformation include *E. coli*, *Bacillus subtilis*, *Salmonella typhimurium*, and various species within the genera
25 *Pseudomonas*, *Streptomyces*, and *Staphylococcus*, although others may also be employed as a matter of choice.

Recombinant RANK may also be expressed in yeast hosts, preferably from the *Saccharomyces* species, such as *S. cerevisiae*. Yeast of other genera, such as *Pichia* or *Kluyveromyces* may also be employed. Yeast vectors will generally contain an origin of
30 replication from the 2 μ yeast plasmid or an autonomously replicating sequence (ARS), promoter, DNA encoding the protein, sequences for polyadenylation and transcription termination and a selection gene. Preferably, yeast vectors will include an origin of replication and selectable marker permitting transformation of both yeast and *E. coli*, e.g., the ampicillin resistance gene of *E. coli* and *S. cerevisiae* *trp1* gene, which provides a
35 selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, and a promoter derived from a highly expressed yeast gene to induce transcription of a structural sequence downstream. The presence of the *trp1* lesion in the yeast host cell genome then

provides an effective environment for detecting transformation by growth in the absence of tryptophan.

Suitable yeast transformation protocols are known to those of skill in the art; an exemplary technique is described by Hinnen et al., *Proc. Natl. Acad. Sci. USA* 75:1929, 1978, selecting for Trp⁺ transformants in a selective medium consisting of 0.67% yeast nitrogen base, 0.5% casamino acids, 2% glucose, 10 µg/ml adenine and 20 µg/ml uracil. Host strains transformed by vectors comprising the ADH2 promoter may be grown for expression in a rich medium consisting of 1% yeast extract, 2% peptone, and 1% glucose supplemented with 80 µg/ml adenine and 80 µg/ml uracil. Derepression of the ADH2 promoter occurs upon exhaustion of medium glucose. Crude yeast supernatants are harvested by filtration and held at 4°C prior to further purification.

Various mammalian or insect cell culture systems can be employed to express recombinant protein. Baculovirus systems for production of heterologous proteins in insect cells are reviewed by Luckow and Summers, *Bio/Technology* 6:47 (1988). Examples of suitable mammalian host cell lines include the COS-7 lines of monkey kidney cells, described by Gluzman (*Cell* 23:175, 1981), and other cell lines capable of expressing an appropriate vector including, for example, CV-1/EBNA (ATCC CRL 10478), L cells, C127, 3T3, Chinese hamster ovary (CHO), HeLa and BHK cell lines. Mammalian expression vectors may comprise nontranscribed elements such as an origin of replication, a suitable promoter and enhancer linked to the gene to be expressed, and other 5' or 3' flanking nontranscribed sequences, and 5' or 3' nontranslated sequences, such as necessary ribosome binding sites, a polyadenylation site, splice donor and acceptor sites, and transcriptional termination sequences.

Purification of Recombinant RANK

Purified RANK, and homologs or analogs thereof are prepared by culturing suitable host/vector systems to express the recombinant translation products of the DNAs of the present invention, which are then purified from culture media or cell extracts. For example, supernatants from systems which secrete recombinant protein into culture media can be first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit.

Following the concentration step, the concentrate can be applied to a suitable purification matrix. For example, a suitable affinity matrix can comprise a counter structure protein or lectin or antibody molecule bound to a suitable support. Alternatively, an anion exchange resin can be employed, for example, a matrix or substrate having pendant diethylaminoethyl (DEAE) groups. The matrices can be acrylamide, agarose, dextran, cellulose or other types commonly employed in protein purification. Alternatively, a cation exchange step can be employed. Suitable cation exchangers include various insoluble

matrices comprising sulfopropyl or carboxymethyl groups. Sulfopropyl groups are preferred. Gel filtration chromatography also provides a means of purifying the inventive proteins.

Affinity chromatography is a particularly preferred method of purifying RANK and homologs thereof. For example, a RANK expressed as a fusion protein comprising an immunoglobulin Fc region can be purified using Protein A or Protein G affinity chromatography. Moreover, a RANK protein comprising an oligomerizing zipper domain may be purified on a resin comprising an antibody specific to the oligomerizing zipper domain. Monoclonal antibodies against the RANK protein may also be useful in affinity chromatography purification, by utilizing methods that are well-known in the art. A ligand may also be used to prepare an affinity matrix for affinity purification of RANK.

Finally, one or more reversed-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify a RANK composition. Some or all of the foregoing purification steps, in various combinations, can also be employed to provide a homogeneous recombinant protein.

Recombinant protein produced in bacterial culture is usually isolated by initial extraction from cell pellets, followed by one or more concentration, salting-out, aqueous ion exchange or size exclusion chromatography steps. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps. Microbial cells employed in expression of recombinant protein can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents.

Fermentation of yeast which express the inventive protein as a secreted protein greatly simplifies purification. Secreted recombinant protein resulting from a large-scale fermentation can be purified by methods analogous to those disclosed by Urdal et al. (*J. Chromatog.* 296:171, 1984). This reference describes two sequential, reversed-phase HPLC steps for purification of recombinant human GM-CSF on a preparative HPLC column.

Protein synthesized in recombinant culture is characterized by the presence of cell components, including proteins, in amounts and of a character which depend upon the purification steps taken to recover the inventive protein from the culture. These components ordinarily will be of yeast, prokaryotic or non-human higher eukaryotic origin and preferably are present in innocuous contaminant quantities, on the order of less than about 1 percent by weight. Further, recombinant cell culture enables the production of the inventive proteins free of other proteins which may be normally associated with the proteins as they are found in nature in the species of origin.

Uses and Administration of RANK Compositions

The present invention provides methods of using therapeutic compositions comprising an effective amount of a protein and a suitable diluent and carrier, and methods for regulating an immune or inflammatory response. The use of RANK in conjunction with soluble cytokine receptors or cytokines, or other immunoregulatory molecules is also contemplated.

For therapeutic use, purified protein is administered to a patient, preferably a human, for treatment in a manner appropriate to the indication. Thus, for example, RANK protein compositions administered to regulate immune function can be given by bolus injection, continuous infusion, sustained release from implants, or other suitable technique. Typically, a therapeutic agent will be administered in the form of a composition comprising purified RANK, in conjunction with physiologically acceptable carriers, excipients or diluents. Such carriers will be nontoxic to recipients at the dosages and concentrations employed.

Ordinarily, the preparation of such protein compositions entails combining the inventive protein with buffers, antioxidants such as ascorbic acid, low molecular weight (less than about 10 residues) polypeptides, proteins, amino acids, carbohydrates including glucose, sucrose or dextrans, chelating agents such as EDTA, glutathione and other stabilizers and excipients. Neutral buffered saline or saline mixed with conspecific serum albumin are exemplary appropriate diluents. Preferably, product is formulated as a lyophilizate using appropriate excipient solutions (e.g., sucrose) as diluents. Appropriate dosages can be determined in trials. The amount and frequency of administration will depend, of course, on such factors as the nature and severity of the indication being treated, the desired response, the condition of the patient, and so forth.

Soluble forms of RANK and other RANK antagonists such as antagonistic monoclonal antibodies can be administered for the purpose of inhibiting RANK-induced induction of NF- κ B activity. NF- κ B is a transcription factor that is utilized extensively by cells of the immune system, and plays a role in the inflammatory response. Thus, inhibitors of RANK signalling will be useful in treating conditions in which signalling through RANK has given rise to negative consequences, for example, toxic or septic shock, or graft-versus-host reactions. They may also be useful in interfering with the role of NF- κ B in cellular transformation. Tumor cells are more responsive to radiation when their NF- κ B is blocked; thus, soluble RANK (or other antagonists of RANK signalling) will be useful as an adjunct therapy for disease characterized by neoplastic cells that express RANK.

The following examples are offered by way of illustration, and not by way of limitation. Those skilled in the art will recognize that variations of the invention embodied in the examples can be made, especially in light of the teachings of the various references cited herein, the disclosures of which are incorporated by reference.

5

EXAMPLE 1

The example describes the identification and isolation of a DNA encoding a novel member of the TNF receptor superfamily. A partial cDNA insert with a predicted open reading frame having some similarity to CD40 (a cell-surface antigen present on the surface of both normal and neoplastic human B cells that has been shown to play an important role in B-cell proliferation and differentiation; Stamenkovic et al., *EMBO J.* 8:1403, 1989), was identified in a database containing sequence information from cDNAs generated from human bone marrow-derived dendritic cells (DC). The insert was excised from the vector by restriction endonuclease digestion, gel purified, labeled with ³²P, and used to hybridize to colony blots generated from a DC cDNA library containing larger cDNA inserts using high stringency hybridization and washing techniques (hybridization in 5xSSC, 50% formamide at 42°C overnight, washing in 0.5xSSC at 63°C); other suitable high stringency conditions are disclosed in Sambrook et al. in Molecular Cloning: A Laboratory Manual, 2nd ed. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; 1989), 9.52-9.55. Initial experiments yielded a clone referred to as 9D-8A (SEQ ID NO:1); subsequent analysis indicated that this clone contained all but the extreme 5' end of a novel cDNA, with predicted intron sequence at the extreme 5' end (nucleotides 1-92 of SEQ ID NO:1). Additional colony hybridizations were performed, and a second clone was isolated. The second clone, referred to as 9D-15C (SEQ ID NO:3), contained the 5' end without intron interruption but not the full 3' end. SEQ ID NO:5 shows the nucleotide and amino acid sequence of a predicted full-length protein based on alignment of the overlapping sequences of SEQ ID NOs:1 and 3.

The encoded protein was designated RANK, for receptor activator of NF-κB. The cDNA encodes a predicted Type 1 transmembrane protein having 616 amino acid residues, with a predicted 24 amino acid signal sequence (the computer predicted cleavage site is after Leu24), a 188 amino acid extracellular domain, a 21 amino acid transmembrane domain, and a 383 amino acid cytoplasmic tail. The extracellular region of RANK displayed significant amino acid homology (38.5% identity, 52.3% similarity) to CD40. A cloning vector (pBluescriptSK-) containing human RANK sequence, designated pBluescript:huRANK (in *E. coli* DH10B), was deposited with the American Type Culture Collection, Rockville, MD (ATCC) on December 20, 1996, under terms of the Budapest Treaty, and given accession number 98285.

EXAMPLE 2

This example describes construction of a RANK DNA construct to express a RANK/Fc fusion protein. A soluble form of RANK fused to the Fc region of human IgG₁ was constructed in the mammalian expression vector pDC409 (USSN 08/571,579). This expression vector encodes the leader sequence of the Cytomegalovirus (CMV) open reading frame R27080 (SEQ ID NO:9), followed by amino acids 33-213 of RANK, followed by a mutated form of the constant domain of human IgG₁ that exhibits reduced affinity for Fc receptors (SEQ ID NO:8; for the fusion protein, the Fc portion of the construct consisted of Arg3 through Lys232). An alternative expression vector encompassing amino acids 1-213 of RANK (using the native leader sequence) followed by the IgG₁ mutein was also prepared. Both expression vectors were found to induce high levels of expression of the RANK/Fc fusion protein in transfected cells.

To obtain RANK/Fc protein, a RANK/Fc expression plasmid is transfected into CV-1/EBNA cells, and supernatants are collected for about one week. The RANK/Fc fusion protein is purified by means well-known in the art for purification of Fc fusion proteins, for example, by protein A sepharose column chromatography according to manufacturer's recommendations (i.e., Pharmacia, Uppsala, Sweden). SDS-polyacrylamide gel electrophoresis analysis indicated that the purified RANK/Fc protein migrated with a molecular weight of ~55kDa in the presence of a reducing agent, and at a molecular weight of ~110kDa in the absence of a reducing agent.

N-terminal amino acid sequencing of the purified protein made using the CMV R27080 leader showed 60% cleavage after Ala20, 20% cleavage after Pro22 and 20% cleavage after Arg28 (which is the Furin cleavage site; amino acid residues are relative to SEQ ID NO:9); N-terminal amino acid analysis of the fusion protein expressed with the native leader showed cleavage predominantly after Gln25 (80% after Gln25 and 20% after Arg23; amino acid residues are relative to SEQ ID NO:6, full-length RANK). Both fusion proteins were able to bind a ligand for RANK in a specific manner (i.e., they bound to the surface of various cell lines such as a murine thymoma cell line, EL4), indicating that the presence of additional amino acids at the N-terminus of RANK does not interfere with its ability to bind RANKL. Moreover, the construct comprising the CMV leader encoded RANK beginning at amino acid 33; thus, a RANK peptide having an N-terminus at an amino acid between Arg23 and Pro33, inclusive, is expected to be able to bind a ligand for RANK in a specific manner.

Other members of the TNF receptor superfamily have a region of amino acids between the transmembrane domain and the ligand binding domain that is referred to as a 'spacer' region, which is not necessary for ligand binding. In RANK, the amino acids between 196 and 213 are predicted to form such a spacer region. Accordingly, a soluble form of RANK that terminates with an amino acid in this region is expected to retain the

ability to bind a ligand for RANK in a specific manner. Preferred C-terminal amino acids for soluble RANK peptides are selected from the group consisting of amino acids 213 and 196 of SEQ ID NO:6, although other amino acids in the spacer region may be utilized as a C-terminus.

EXAMPLE 3

This example illustrates the preparation of monoclonal antibodies against RANK. Preparations of purified recombinant RANK, for example, or transfected cells expressing high levels of RANK, are employed to generate monoclonal antibodies against RANK using conventional techniques, such as those disclosed in U.S. Patent 4,411,993. DNA encoding RANK can also be used as an immunogen, for example, as reviewed by Pardoll and Beckerleg in *Immunity* 3:165, 1995. Such antibodies are likely to be useful in interfering with RANK-induced signaling (antagonistic or blocking antibodies) or in inducing a signal by cross-linking RANK (agonistic antibodies), as components of diagnostic or research assays for RANK or RANK activity, or in affinity purification of RANK.

To immunize rodents, RANK immunogen is emulsified in an adjuvant (such as complete or incomplete Freund's adjuvant, alum, or another adjuvant, such as Ribi adjuvant R700 (Ribi, Hamilton, MT), and injected in amounts ranging from 10-100 µg subcutaneously into a selected rodent, for example, BALB/c mice or Lewis rats. DNA may be given intradermally (Raz et al., *Proc. Natl. Acad. Sci. USA* 91:9519, 1994) or intramuscularly (Wang et al., *Proc. Natl. Acad. Sci. USA* 90:4156, 1993); saline has been found to be a suitable diluent for DNA-based antigens. Ten days to three weeks days later, the immunized animals are boosted with additional immunogen and periodically boosted thereafter on a weekly, biweekly or every third week immunization schedule.

Serum samples are periodically taken by retro-orbital bleeding or tail-tip excision for testing by dot-blot assay (antibody sandwich), ELISA (enzyme-linked immunosorbent assay), immunoprecipitation, or other suitable assays, including FACS analysis. Following detection of an appropriate antibody titer, positive animals are given an intravenous injection of antigen in saline. Three to four days later, the animals are sacrificed, splenocytes harvested, and fused to a murine myeloma cell line (e.g., NS1 or preferably Ag 8.653 [ATCC CRL 1580]). Hybridoma cell lines generated by this procedure are plated in multiple microtiter plates in a selective medium (for example, one containing hypoxanthine, aminopterin, and thymidine, or HAT) to inhibit proliferation of non-fused cells, myeloma-myeloma hybrids, and splenocyte-splenocyte hybrids.

Hybridoma clones thus generated can be screened by ELISA for reactivity with RANK, for example, by adaptations of the techniques disclosed by Engvall et al., *Immunochem.* 8:871 (1971) and in U.S. Patent 4,703,004. A preferred screening

technique is the antibody capture technique described by Beckman et al., *J. Immunol.* 144:4212 (1990). Positive clones are then injected into the peritoneal cavities of syngeneic rodents to produce ascites containing high concentrations (>1 mg/ml) of anti-RANK monoclonal antibody. The resulting monoclonal antibody can be purified by ammonium sulfate precipitation followed by gel exclusion chromatography. Alternatively, affinity chromatography based upon binding of antibody to protein A or protein G can also be used, as can affinity chromatography based upon binding to RANK protein.

Monoclonal antibodies were generated using RANK/Fc fusion protein as the immunogen. These reagents were screened to confirm reactivity against the RANK protein. Using the methods described herein to monitor the activity of the mAbs, both blocking (i.e., antibodies that bind RANK and inhibit binding of a ligand to RANK) and non-blocking (i.e., antibodies that bind RANK and do not inhibit ligand binding) were isolated.

EXAMPLE 4

This example illustrates the induction of NF- κ B activity by RANK in 293/EBNA cells (cell line was derived by transfection of the 293 cell line with a gene encoding Epstein-Barr virus nuclear antigen-1 (EBNA-1) that constitutively express EBNA-1 driven from human CMV immediate-early enhancer/promoter). Activation of NF- κ B activity was measured in 293/EBNA cells essentially as described by Yao et al. (*Immunity* 3:811, 1995). Nuclear extracts were prepared and analyzed for NF- κ B activity by a gel retardation assay using a 25 base pair oligonucleotide spanning the NF- κ B binding sites. Two million cells were seeded into 10 cm dishes two days prior to DNA transfection and cultured in DMEM-F12 media containing 2.5% FBS (fetal bovine serum). DNA transfections were performed as described herein for the IL-8 promoter/reporter assays.

Nuclear extracts were prepared by solubilization of isolated nuclei with 400 mM NaCl (Yao et al., *supra*). Oligonucleotides containing an NF- κ B binding site were annealed and endlabeled with 32 P using T4 DNA polynucleotide kinase. Mobility shift reactions contained 10 μ g of nuclear extract, 4 μ g of poly(dI-dC) and 15,000 cpm labeled double-stranded oligonucleotide and incubated at room temperature for 20 minutes. Resulting protein-DNA complexes were resolved on a 6% native polyacrylamide gel in 0.25 X Tris-borate-EDTA buffer.

Overexpression of RANK resulted in induction of NF- κ B activity as shown by an appropriate shift in the mobility of the radioactive probe on the gel. Similar results were observed when RANK was triggered by a ligand that binds RANK and transduces a signal to cells expressing the receptor (i.e., by co-transfecting cells with human RANK and murine RANKL DNA; see Example 7 below), and would be expected to occur when triggering is done with agonistic antibodies.

EXAMPLE 5

This example describes a gene promoter/reporter system based on the human Interleukin-8 (IL-8) promoter used to analyze the activation of gene transcription in vivo. The induction of human IL-8 gene transcription by the cytokines Interleukin-1 (IL-1) or tumor necrosis factor-alpha (TNF- α) is known to be dependent upon intact NF- κ B and NF-IL-6 transcription factor binding sites. Fusion of the cytokine-responsive IL-8 promoter with a cDNA encoding the murine IL-4 receptor (mIL-4R) allows measurement of promoter activation by detection of the heterologous reporter protein (mIL-4R) on the cell surface of transfected cells.

Human kidney epithelial cells (293/EBNA) are transfected (via the DEAE/DEXTRAN method) with plasmids encoding: 1). the reporter/promoter construct (referred to as pIL-8rep), and 2). the cDNA(s) of interest. DNA concentrations are always kept constant by the addition of empty vector DNA. The 293/EBNA cells are plated at a density of 2.5×10^4 cells/ml (3 ml/ well) in a 6 well plate and incubated for two days prior to transfection. Two days after transfection, the mIL-4 receptor is detected by a radioimmunoassay (RIA) described below.

In one such experiment, the 293/EBNA cells were co-transfected with DNA encoding RANK and with DNA encoding RANKL (see Example 7 below). Co-expression of this receptor and its counterstructure by cells results in activation of the signaling process of RANK. For such co-transfection studies, the DNA concentration/well for the DEAE transfection were as follows: 40 ng of pIL-8rep [pBluescriptSK⁻ vector (Stratagene)]; 0.4 ng CD40 (DNA encoding CD40, a control receptor; pCDM8 vector); 0.4 ng RANK (DNA encoding RANK; pDC409 vector), and either 1-50 ng CD40L (DNA encoding the ligand for CD40, which acts as a positive control when co-transfected with CD40 and as a negative control when co-transfected with RANK; in pDC304) or RANKL (DNA encoding a ligand for RANK; in pDC406). Similar experiments can be done using soluble RANKL or agonistic antibodies to RANK to trigger cells transfected with RANK.

For the mIL-4R-specific RIA, a monoclonal antibody reactive with mIL-4R is labeled with ^{125}I via a Chloramine T conjugation method; the resulting specific activity is typically 1.5×10^{16} cpm/nmol. After 48 hours, transfected cells are washed once with media (DMEM/F12 5% FBS). Non-specific binding sites are blocked by the addition of pre-warmed binding media containing 5% non-fat dry milk and incubation at 37°C/5% CO₂ in a tissue culture incubator for one hour. The blocking media is decanted and binding buffer containing ^{125}I anti-mIL-4R (clone M1; rat IgG1) is added to the cells and incubated with rocking at room temperature for 1 hour. After incubation of the cells with the radio-labeled antibody, cells are washed extensively with binding buffer (2X) and twice with

phosphate-buffered saline (PBS). Cells are lysed in 1 ml of 0.5M NaOH, and total radioactivity is measured with a gamma counter.

Using this assay, 293/EBNA co-transfected with DNAs encoding RANK demonstrated transcriptional activation, as shown by detection of muIL-4R on the cell surface. Overexpression of RANK resulted in transcription of muIL-4R, as did triggering of the RANK by RANKL. Similar results are observed when RANK is triggered by agonistic antibodies.

EXAMPLE 6

This example illustrates the association of RANK with TRAF proteins. Interaction of RANK with cytoplasmic TRAF proteins was demonstrated by co-immunoprecipitation assays essentially as described by Hsu et al. (*Cell* 84:299; 1996). Briefly, 293/EBNA cells were co-transfected with plasmids that direct the synthesis of RANK and epitope-tagged (FLAG®; SEQ ID NO:7) TRAF2 or TRAF3. Two days after transfection, surface proteins were labeled with biotin-ester, and cells were lysed in a buffer containing 0.5% NP-40. RANK and proteins associated with this receptor were immunoprecipitated with anti-RANK, washed extensively, resolved by electrophoretic separation on a 6-10% SDS polyacrylamide gel and electrophoretically transferred to a nitrocellulose membrane for Western blotting. The association of TRAF2 and TRAF3 proteins with RANK was visualized by probing the membrane with an antibody that specifically recognizes the FLAG® epitope. TRAFs 2 and 3 did not immunoprecipitate with anti-RANK in the absence of RANK expression.

EXAMPLE 7

This example describes isolation of a ligand for RANK, referred to as RANKL, by direct expression cloning. The ligand was cloned essentially as described in USSN 08/249,189, filed May 24, 1994 (the relevant disclosure of which is incorporated by reference herein), for CD40L. Briefly, a library was prepared from a clone of a mouse thymoma cell line EL-4 (ATCC TIB 39), called EL-40.5, derived by sorting five times with biotinylated CD40/Fc fusion protein in a FACS (fluorescence activated cell sorter). The cDNA library was made using standard methodology; the plasmid DNA was isolated and transfected into sub-confluent CV1-EBNA cells using a DEAE-dextran method. Transfectants were screened by slide autoradiography for expression of RANKL using a two-step binding method with RANK/Fc fusion protein as prepared in Example 2 followed by radioiodinated goat anti-human IgG antibody.

A clone encoding a protein that specifically bound RANK was isolated and sequenced; the clone was referred to as 11H. An expression vector containing murine RANKL sequence, designated pDC406:muRANK-L (in *E. coli* DH10B), was deposited

with the American Type Culture Collection, Rockville, MD (ATCC) on December 20, 1996, under terms of the Budapest Treaty, and given accession number 98284. The nucleotide sequence and predicted amino acid sequence of this clone are illustrated in SEQ ID NO:10. This clone did not contain an initiator methionine; additional, full-length clones
5 were obtained from a 7B9 library (prepared substantially as described in US patent 5,599,905, issued February 4, 1997); the 5' region was found to be identical to that of human RANKL as shown in SEQ ID NO: 12, amino acids 1 through 22, except for substitution of a Gly for a Thr at residue 9.

This ligand is useful for assessing the ability of RANK to bind RANKL by a
10 number of different assays. For example, transfected cells expressing RANKL can be used in a FACS assay (or similar assay) to evaluate the ability of soluble RANK to bind RANKL. Moreover, soluble forms of RANKL can be prepared and used in assays that are known in the art (i.e., ELISA or BIAcore assays essentially as described in USSN 08/249,189, filed May 24, 1994). RANKL is also useful in affinity purification of RANK,
15 and as a reagent in methods to measure the levels of RANK in a sample. Soluble RANKL is also useful in inducing NF- κ B activation and thus protecting cells that express RANK from apoptosis.

EXAMPLE 8

This example describes the isolation of a human RANK ligand (RANKL) using a
20 PCR-based technique. Murine RANK ligand-specific oligonucleotide primers were used in PCR reactions using human cell line-derived first strand cDNAs as templates. Primers corresponded to nucleotides 478-497 and to the complement of nucleotides 858-878 of murine RANK ligand (SEQ ID NO:10). An amplified band approximately 400 bp in length from one reaction using the human epidermoid cell line KB (ATCC CCL-17) was gel
25 purified, and its nucleotide sequence determined; the sequence was 85% identical to the corresponding region of murine RANK ligand, confirming that the fragment was from human RANKL.

To obtain full-length human RANKL cDNAs, two human RANKL-specific oligonucleotides derived from the KB PCR product nucleotide sequence were radiolabeled
30 and used as hybridization probes to screen a human PBL cDNA library prepared in lambda gt10 (Stratagene, La Jolla, CA), substantially as described in US patent 5,599,905, issued February 4, 1997. Several positive hybridizing plaques were identified and purified, their inserts subcloned into pBluescript SK⁻ (Stratagene, La Jolla, CA), and their nucleotide sequence determined. One isolate, PBL3, was found to encode most of the predicted
35 human RANKL, but appeared to be missing approximately 200 bp of 5' coding region. A second isolate, PBL5 was found to encode much of the predicted human RANKL, including the entire 5' end and an additional 200 bp of 5' untranslated sequence.

The 5' end of PBL5 and the 3' end of PBL3 were ligated together to form a full length cDNA encoding human RANKL. The nucleotide and predicted amino acid sequence of the full-length human RANK ligand is shown in SEQ ID NO:12. Human RANK ligand shares 83% nucleotide and 84% amino acid identity with murine RANK ligand. A plasmid vector containing human RANKL sequence, designated pBluescript:huRANK-L (in *E. coli* DH10B), was deposited with the American Type Culture Collection, Rockville, MD (ATCC) on March 11, 1997 under terms of the Budapest Treaty, and given accession number 98354.

Murine and human RANKL are Type 2 transmembrane proteins. Murine RANKL contains a predicted 48 amino acid intracellular domain, 21 amino acid transmembrane domain and 247 amino acid extracellular domain. Human RANKL contains a predicted 47 amino acid intracellular domain, 21 amino acid transmembrane domain and 249 amino acid extracellular domain.

EXAMPLE 9

This example describes the chromosomal mapping of human RANK using PCR-based mapping strategies. Initial human chromosomal assignments were made using RANK and RANKL-specific PCR primers and a BIOS Somatic Cell Hybrid PCRable DNA kit from BIOS Laboratories (New Haven, CT), following the manufacturer's instructions. RANK mapped to human chromosome 18; RANK ligand mapped to human chromosome 13. More detailed mapping was performed using a radiation hybrid mapping panel Genebridge 4 Radiation Hybrid Panel (Research Genetics, Huntsville, AL; described in Walter, MA et al., *Nature Genetics* 7:22-28, 1994). Data from this analysis was then submitted electronically to the MIT Radiation Hybrid Mapper (URL: <http://www-genome.wi.mit.edu/cgi-bin/contig/rhmapper.pl>) following the instructions contained therein. This analysis yielded specific genetic marker names which, when submitted electronically to the NCBI Entrez browser (URL: <http://www3.ncbi.nlm.nih.gov/htbin-post/Entrez/query?db=c&form=0>), yielded the specific map locations. RANK mapped to chromosome 18q22.1, and RANKL mapped to chromosome 13q14.

EXAMPLE 10

This example illustrates the preparation of monoclonal antibodies against RANKL. Preparations of purified recombinant RANKL, for example, or transfected cells expressing high levels of RANKL, are employed to generate monoclonal antibodies against RANKL using conventional techniques, such as those disclosed in US Patent 4,411,993. DNA encoding RANKL can also be used as an immunogen, for example, as reviewed by Pardoll and Beckerleg in *Immunity* 3:165, 1995. Such antibodies are likely to be useful in interfering with RANKL signaling (antagonistic or blocking antibodies), as components of

diagnostic or research assays for RANKL or RANKL activity, or in affinity purification of RANKL.

To immunize rodents, RANKL immunogen is emulsified in an adjuvant (such as complete or incomplete Freund's adjuvant, alum, or another adjuvant, such as Ribi adjuvant R700 (Ribi, Hamilton, MT), and injected in amounts ranging from 10-100 µg subcutaneously into a selected rodent, for example, BALB/c mice or Lewis rats. DNA may be given intradermally (Raz et al., *Proc. Natl. Acad. Sci. USA* 91:9519, 1994) or intamuscularly (Wang et al., *Proc. Natl. Acad. Sci. USA* 90:4156, 1993); saline has been found to be a suitable diluent for DNA-based antigens. Ten days to three weeks days later, the immunized animals are boosted with additional immunogen and periodically boosted thereafter on a weekly, biweekly or every third week immunization schedule.

Serum samples are periodically taken by retro-orbital bleeding or tail-tip excision for testing by dot-blot assay (antibody sandwich), ELISA (enzyme-linked immunosorbent assay), immunoprecipitation, or other suitable assays, including FACS analysis. Following detection of an appropriate antibody titer, positive animals are given an intravenous injection of antigen in saline. Three to four days later, the animals are sacrificed, splenocytes harvested, and fused to a murine myeloma cell line (e.g., NS1 or preferably Ag 8.653 [ATCC CRL 1580]). Hybridoma cell lines generated by this procedure are plated in multiple microtiter plates in a selective medium (for example, one containing hypoxanthine, aminopterin, and thymidine, or HAT) to inhibit proliferation of non-fused cells, myeloma-myeloma hybrids, and splenocyte-splenocyte hybrids.

Hybridoma clones thus generated can be screened by ELISA for reactivity with RANKL, for example, by adaptations of the techniques disclosed by Engvall et al., *Immunochem.* 8:871 (1971) and in US Patent 4,703,004. A preferred screening technique is the antibody capture technique described by Beckman et al., *J. Immunol.* 144:4212 (1990). Positive clones are then injected into the peritoneal cavities of syngeneic rodents to produce ascites containing high concentrations (>1 mg/ml) of anti-RANK monoclonal antibody. The resulting monoclonal antibody can be purified by ammonium sulfate precipitation followed by gel exclusion chromatography. Alternatively, affinity chromatography based upon binding of antibody to protein A or protein G can also be used, as can affinity chromatography based upon binding to RANKL protein. Using the methods described herein to monitor the activity of the mAbs, both blocking (i.e., antibodies that bind RANKL and inhibit binding to RANK) and non-blocking (i.e., antibodies that bind RANKL and do not inhibit binding) are isolated.

EXAMPLE 11

This example demonstrates that RANK expression can be up-regulated. Human peripheral blood T cells were purified by flow cytometry sorting or by negative selection using antibody coated beads, and activated with anti-CD3 (OKT3, Dako) coated plates or phytohemagglutinin in the presence or absence of various cytokines, including Interleukin-4 (IL-4), Transforming Growth Factor- β (TGF- β) and other commercially available cytokines (IL-1- α , IL-2, IL-3, IL-6, IL-7, IL-8, IL-10, IL-12, IL-15, IFN- γ , TNF- α). Expression of RANK was evaluated by FACS in a time course experiment for day 2 to day 8, using a mouse monoclonal antibody mAb144 (prepared as described in Example 3), as shown in the table below. Results are expressed as '+' to '++++' referring to the relative increase in intensity of staining with anti-RANK. Double labeling experiments using both anti-RANK and anti-CD8 or anti-CD4 antibodies were also performed.

Table 1: Upregulation of RANK by Cytokines

Cytokine (concentration)	Results:
IL-4 (50 ng/ml)	+
TGF- β (5 ng/ml)	+ to ++
IL-4 (50 ng/ml) +TGF- β (5 ng/ml)	++++
IL-1- α (10ng/ml)	-
IL-2 (20ng/ml)	-
IL-3 (25ng/ml)	-
IL-7 (20ng/ml)	-
IL-8 (10ng/ml)	-
IL-10 (50ng/ml)	-
IL-12 (10ng/ml)	-
IL-15 (10ng/ml)	-
IFN- γ (100U/ml)	-
TNF- α (10ng/ml)	-

Of the cytokines tested, IL-4 and TGF- β increased the level of RANK expression on both CD8+ cytotoxic and CD4+ helper T cells from day 4 to day 8. The combination of IL-4 and TGF- β acted synergistically to upregulate expression of this receptor on activated T cells. This particular combination of cytokines is secreted by suppressor T cells, and is believed to be important in the generation of tolerance (reviewed in Mitchison and Sieper, *Z. Rheumatol.* 54:141, 1995), implicating the interaction of RANK in regulation of an immune response towards either tolerance or induction of an active immune response.

EXAMPLE 12

This example illustrates the influence of RANK.Fc and hRANKL on activated T cell growth. The addition of TGF β to anti-CD3 activated human peripheral blood T lymphocytes induces proliferation arrest and ultimately death of most lymphocytes within the first few days of culture. We tested the effect of RANK:RANKL interactions on TGF β -treated T cells by adding RANK.Fc or soluble human RANKL to T cell cultures.

Human peripheral blood T cells (7×10^5 PBT) were cultured for six days on anti-CD3 (OKT3, 5 μ g/ml) and anti-Flag (M1, 5 μ g/ml) coated 24 well plates in the presence of TGF β (1ng/ml) and IL-4 (10ng/ml), with or without recombinant FLAG-tagged soluble hRANKL (1 μ g/ml) or RANK.Fc (10 μ g/ml). Viable T cell recovery was determined by triplicate trypan blue countings.

The addition of RANK.Fc significantly reduced the number of viable T cells recovered after six days, whereas soluble RANKL greatly increased the recovery of viable T cells (Figure 1). Thus, endogenous or exogenous RANKL enhances the number of viable T cells generated in the presence of TGF β . TGF β , along with IL-4, has been implicated in immune response regulation when secreted by the T_H3/regulatory T cell subset. These T cells are believed to mediate bystander suppression of effector T cells. Accordingly, RANK and its ligand may act in an auto/paracrine fashion to influence T cell tolerance. Moreover, TGF β is known to play a role in the evasion of the immune system effected by certain pathogenic or opportunistic organisms. In addition to playing a role in the development of tolerance, RANK may also play a role in immune system evasion by pathogens.

EXAMPLE 13

This example illustrates the influence of the interaction of RANK on CD1a⁺ dendritic cells (DC). Functionally mature dendritic cells (DC) were generated *in vitro* from CD34⁺ bone marrow (BM) progenitors. Briefly, human BM cells from normal healthy volunteers were density fractionated using Ficoll medium and CD34⁺ cells immunoaffinity isolated using an anti-CD34 matrix column (Ceprate, CellPro). The CD34⁺ BM cells were then cultured in human GM-CSF (20 ng/ml), human IL-4 (20 ng/ml), human TNF- α (20 ng/ml), human CHO-derived Flt3L (FL; 100 ng/ml) in Super McCoy's medium supplemented with 10% fetal calf serum in a fully humidified 37°C incubator (5% CO₂) for 14 days. CD1a⁺, HLA-DR⁺ DC were then sorted using a FACStar Plus™, and used for biological evaluation of RANK.

On human CD1a⁺ DC derived from CD34⁺ bone marrow cells, only a subset (20-30%) of CD1a⁺ DC expressed RANK at the cell surface as assessed by flow cytometric

analysis. However, addition of CD40L to the DC cultures resulted in RANK surface expression on the majority of CD1a⁺ DC. CD40L has been shown to activate DC by enhancing *in vitro* cluster formation, inducing DC morphological changes and upregulating HLA-DR, CD54, CD58, CD80 and CD86 expression

5 Addition of RANKL to DC cultures significantly increased the degree of DC aggregation and cluster formation above control cultures, similar to the effects seen with CD40L (Figure 2). Sorted human CD1a⁺ DC were cultured in a cytokine cocktail (GM-CSF, IL-4, TNF- α and FL) (upper left panel), in cocktail plus CD40L (1 μ g/ml) (upper right), in cocktail plus RANKL (1 μ g/ml) (lower left), or in cocktail plus heat inactivated
10 (Δ H) RANKL (1 μ g/ml) (lower right) in 24-well flat bottomed culture plates in 1 ml culture media for 48-72 hours and then photographed using an inversion microscope. An increase in DC aggregation and cluster formation above control cultures was not evident when heat inactivated RANKL was used, indicating that this effect was dependent on biologically active protein. However, initial phenotypic analysis of adhesion molecule expression
15 indicated that RANKL-induced clustering was not due to increased levels of CD2, CD11a, CD54 or CD58.

The addition of RANKL to CD1a⁺ DC enhanced their allo-stimulatory capacity in a mixed lymphocyte reaction (MLR) by at least 3- to 10-fold, comparable to CD40L-cultured DC (Figure 3). Allogeneic T cells (1x10⁵) were incubated with varying numbers of
20 irradiated (2000 rad) DC cultured as indicated above for Figure 2 in 96-well round bottomed culture plates in 0.2 ml culture medium for four days. The cultures were pulsed with 0.5 mCi [³H]-thymidine for eight hours and the cells harvested onto glass fiber sheets for counting on a gas phase β counter. The background counts for either T cells or DC cultured alone were <100 cpm. Values represent the mean \pm SD of triplicate cultures. Heat
25 inactivated RANKL had no effect. DC allo-stimulatory activity was not further enhanced when RANKL and CD40L were used in combination, possibly due to DC functional capacity having reached a maximal level with either cytokine alone. Neither RANKL nor CD40L enhanced the *in vitro* growth of DC over the three day culture period. Unlike CD40L, RANKL did not significantly increase the levels of HLA-DR expression nor the
30 expression of CD80 or CD86.

RANKL can enhance DC cluster formation and functional capacity without modulating known molecules involved in cell adhesion (CD18, CD54), antigen presentation (HLA-DR) or costimulation (CD86), all of which are regulated by CD40/CD40L signaling. The lack of an effect on the expression of these molecules
35 suggests that RANKL may regulate DC function via an alternate pathway(s) distinct from CD40/CD40L. Given that CD40L regulates RANK surface expression on *in vitro*-generated DC and that CD40L is upregulated on activated T cells during DC-T cell

interactions, RANK and its ligand may form an important part of the activation cascade that is induced during DC-mediated T cell expansion. Furthermore, culture of DC in RANKL results in decreased levels of CD1b/c expression, and increased levels of CD83. Both of these molecules are similarly modulated during DC maturation by CD40L (Caux et al. *J. Exp. Med.* 180:1263; 1994), indicating that RANKL induces DC maturation.

Dendritic cells are referred to as "professional" antigen presenting cells, and have a high capacity for sensitizing MHC-restricted T cells. There is growing interest in using dendritic cells *ex vivo* as tumor or infectious disease vaccine adjuvants (see, for example, Romani, et al., *J. Exp. Med.*, 180:83, 1994). Therefore, an agent such as RANKL that induces DC maturation and enhances the ability of dendritic cells to stimulate an immune response is likely to be useful in immunotherapy of various diseases.

EXAMPLE 14

This example describes the isolation of the murine homolog of RANK, referred to as muRANK. MuRANK was isolated by a combination of cross-species PCR and colony hybridization. The conservation of Cys residues in the Cys-rich pseudorepeats of the extracellular domains of TNFR superfamily member proteins was exploited to design human RANK-based PCR primers to be used on murine first strand cDNAs from various sources. Both the sense upstream primer and the antisense downstream primer were designed to have their 3' ends terminate within Cys residues.

The upstream sense primer encoded nucleotides 272-295 of SEQ ID NO:5 (region encoding amino acids 79-86); the downstream antisense primer encoded the complement of nucleotides 409-427 (region encoding amino acids 124-130). Standard PCR reactions were set up and run, using these primers and first strand cDNAs from various murine cell line or tissue sources. Thirty reaction cycles of 94°C for 30 seconds, 50°C for 30 seconds, and 72°C for 20 seconds were run. PCR products were analyzed by electrophoresis, and specific bands were seen in several samples. The band from one sample was gel purified and DNA sequencing revealed that the sequence between the primers was approximately 85% identical to the corresponding human RANK nucleotide sequence.

A plasmid based cDNA library prepared from the murine fetal liver epithelium line FLE18 (one of the cell lines identified as positive in the PCR screen) was screened for full-length RANK cDNAs using murine RANK-specific oligonucleotide probes derived from the murine RANK sequence determined from sequencing the PCR product. Two cDNAs, one encoding the 5' end and one encoding the 3' end of full-length murine RANK (based on sequence comparison with the full-length human RANK) were recombined to generate a full-length murine RANK cDNA. The nucleotide and amino acid sequence of muRANK are shown in SEQ ID Nos:14 and 15.

The cDNA encodes a predicted Type 1 transmembrane protein having 625 amino acid residues, with a predicted 30 amino acid signal sequence, a 184 amino acid extracellular domain, a 21 amino acid transmembrane domain, and a 390 amino acid cytoplasmic tail. The extracellular region of muRANK displayed significant amino acid
5 homology (69.7% identity, 80.8% similarity) to huRANK. Those of skill in the art will recognize that the actual cleavage site can be different from that predicted by computer; accordingly, the N-terminal of RANK may be from amino acid 25 to amino acid 35.

Other members of the TNF receptor superfamily have a region of amino acids between the transmembrane domain and the ligand binding domain that is referred to as a
10 'spacer' region, which is not necessary for ligand binding. In muRANK, the amino acids between 197 and 214 are predicted to form such a spacer region. Accordingly, a soluble form of RANK that terminates with an amino acid in this region is expected to retain the ability to bind a ligand for RANK in a specific manner. Preferred C-terminal amino acids for soluble RANK peptides are selected from the group consisting of amino acids 214, and
15 197 of SEQ ID NO:14, although other amino acids in the spacer region may be utilized as a C-terminus.

EXAMPLE 15

This example illustrates the preparation of several different soluble forms of RANK
20 and RANKL. Standard techniques of restriction enzyme cutting and ligation, in combination with PCR-based isolation of fragments for which no convenient restriction sites existed, were used. When PCR was utilized, PCR products were sequenced to ascertain whether any mutations had been introduced; no such mutations were found.

In addition to the huRANK/Fc described in Example 2, another RANK/Fc fusion
25 protein was prepared by ligating DNA encoding amino acids 1-213 of SEQ ID NO:6, to DNA encoding amino acids 3-232 of the Fc mutein described previously (SEQ ID NO:8). A similar construct was prepared for murine RANK, ligating DNA encoding amino acids 1-213 of full-length murine RANK (SEQ ID NO:15) to DNA encoding amino acids 3-232 of the Fc mutein (SEQ ID NO:8).

A soluble, tagged, poly-His version of huRANKL was prepared by ligating DNA
30 encoding the leader peptide from the immunoglobulin kappa chain (SEQ ID NO:16) to DNA encoding a short version of the FLAG™ tag (SEQ ID NO:17), followed by codons encoding Gly Ser, then a poly-His tag (SEQ ID NO:18), followed by codons encoding Gly Thr Ser, and DNA encoding amino acids 138-317 of SEQ ID NO:13. A soluble, poly-His
35 tagged version of murine RANKL was prepared by ligating DNA encoding the CMV leader (SEQ ID NO:9) to codons encoding Arg Thr Ser, followed by DNA encoding poly-His (SEQ ID NO:18) followed by DNA encoding amino acids 119-294 of SEQ ID NO:11.

A soluble, oligomeric form of huRANKL was prepared by ligating DNA encoding the CMV leader (SEQ ID NO:9) to a codon encoding Asp followed by DNA ending a trimer-former "leucine" zipper (SEQ ID NO:19), then by codons encoding Thr Arg Ser followed by amino acids 138-317 of SEQ ID NO:13.

5 These and other constructs are prepared by routine experimentation. The various DNAs are then inserted into a suitable expression vector, and expressed. Particularly preferred expression vectors are those which can be used in mammalian cells. For example, pDC409 and pDC304, described herein, are useful for transient expression. For stable transfection, the use of CHO cells is preferred; several useful vectors are described in
10 USSN 08/785,150, now allowed, for example, one of the 2A5-3 λ -derived expression vectors discussed therein.

EXAMPLE 16

15 This example demonstrates that RANKL expression can be up-regulated on murine T cells. Cells were obtained from mesenteric lymph nodes of C57BL/6 mice, and activated with anti-CD3 coated plates, Concanavalin A (ConA) or phorbol myristate acetate in combination with ionomycin (anti-CD3: 500A2; Immunex Corporation, Seattle WA; ConA, PMA, ionomycin, Sigma, St. Louis, MO) substantially as described herein, and cultured from about 2 to 5 days. Expression of RANKL was evaluated in a three color analysis by
20 FACS, using antibodies to the T cell markers CD4, CD8 and CD45RB, and RANK/Fc, prepared as described herein.

RANKL was not expressed on unstimulated murine T cells. T cells stimulated with either anti-CD3, ConA, or PMA/ionomycin, showed differential expression of RANKL: CD4⁺/CD45RB^{Lo} and CD4⁺/CD45RB^{Hi} cells were positive for RANKL, but CD8⁺ cells
25 were not. RANKL was not observed on B cells, similar to results observed with human cells.

EXAMPLE 17

30 This example illustrates the effects of murine RANKL on cell proliferation and activation. Various cells or cell lines representative of cells that play a role in an immune response (murine spleen, thymus and lymphnode) were evaluated by culturing them under conditions promoting their viability, in the presence or absence of RANKL. RANKL did not stimulate any of the tested cells to proliferate. One cell line, a macrophage cell line referred to as RAW 264.7 (ATCC accession number TIB 71) exhibited some signs of
35 activation.

RAW cells constitutively produce small amounts of TNF- α . Incubation with either human or murine RANKL enhanced production of TNF- α by these cells in a dose

dependent manner. The results were not due to contamination of RANKL preparations with endotoxin, since boiling RANKL for 10 minutes abrogated TNF- α production, whereas a similar treatment of purified endotoxin (LPS) did not affect the ability of the LPS to stimulate TNF- α production. Despite the fact that RANKL activated the macrophage cell line RAW T64.7 for TNF- α production, neither human RANKL nor murine RANKL stimulated nitric oxide production by these cells.

EXAMPLE 18

This example illustrates the effects of murine RANKL on growth and development of the thymus in fetal mice. Pregnant mice were injected with 1 mg of RANK/Fc or vehicle control protein (murine serum albumin; MSA) on days 13, 16 and 19 of gestation. After birth, the neonates continued to be injected with RANK/Fc intraperitoneally (IP) on a daily basis, beginning at a dose of 1 μ g, and doubling the dose about every four days, for a final dosage of 4 μ g. Neonates were taken at days 1, 8 and 15 post birth, their thymuses and spleens harvested and examined for size, cellularity and phenotypic composition.

A slight reduction in thymic size at day 1 was observed in the neonates born to the female injected with RANK/Fc; a similar decrease in size was not observed in the control neonates. At day 8, thymic size and cellularity were reduced by about 50% in the RANK/Fc-treated animals as compared to MSA treated mice. Phenotypic analysis demonstrated that the relative proportions of different T cell populations in the thymus were the same in the RANK/Fc mice as the control mice, indicating that the decreased cellularity was due to a global depression in the number of thymic T cells as opposed to a decrease in a specific population(s). The RANK/Fc-treated neonates were not significantly different from the control neonates at day 15 with respect to either size, cellularity or phenotype of thymic cells. No significant differences were observed in spleen size, cellularity or composition at any of the time points evaluated. The difference in cellularity on day 8 and not on day 15 may suggest that RANK/Fc may assert its effect early in thymic development.

EXAMPLE 19

This example demonstrates that the C-terminal region of the cytoplasmic domain of RANK is important for binding of several different TRAF proteins. RANK contains at least two recognizable PXQX(X)T motifs that are likely TRAF docking sites. Accordingly, the importance of various regions of the cytoplasmic domain of RANK for TRAF binding was evaluated. A RANK/GST fusion protein was prepared substantially as described in Smith and Johnson, *Gene* 67:31 (1988), and used in the preparation of various truncations as described below.

Comparison of the nucleotide sequence of murine and human RANK indicated that there were several conserved regions that could be important for TRAF binding. Accordingly, a PCR-based technique was developed to facilitate preparation of various C-terminal truncations that would retain the conserved regions. PCR primers were designed to introduce a stop codon and restriction enzyme site at selected points, yielding the truncations described in Table 1 below. Sequencing confirmed that no undesired mutations had been introduced in the constructs.

Radio-labeled (³⁵S-Met, Cys) TRAF proteins were prepared by *in vitro* translation using a commercially available reticulocyte lysate kit according to manufacturer's instructions (Promega). Truncated GST fusion proteins were purified substantially as described in Smith and Johnson (supra). Briefly, *E. coli* were transfected with an expression vector encoding a fusion protein, and induced to express the protein. The bacteria were lysed, insoluble material removed, and the fusion protein isolated by precipitation with glutathione-coated beads (Sepahrose 4B, Pharmacia, Uppsala Sweden)

The beads were washed, and incubated with various radiolabeled TRAF proteins. After incubation and wash steps, the fusion protein/TRAF complexes were removed from the beads by boiling in 0.1% SDS + β -mercaptoethanol, and loaded onto 12% SDS gels (Novex). The gels were subjected to autoradiography, and the presence or absence of radiolabeled material recorded. The results are shown in Table 2 below.

Table 2: Binding of Various TRAF Proteins to the Cytoplasmic Domain of RANK

C terminal Truncations:	E206-S339	E206-Y421	E206-M476	E206-G544	Full length
TRAF1	-	-	-	-	++
TRAF2	-	-	-	-	++
TRAF3	-	-	-	-	++
TRAF4	-	-	-	-	-
TRAF5	-	-	-	-	+
TRAF6	-	+	+	+	++

These results indicate that TRAF1, TRAF2, TRAF3, TRAF 5 and TRAF6 bind to the most distal portion of the RANK cytoplasmic domain (between amino-acid G544 and A616). TRAF6 also has a binding site between S339 and Y421. In this experiment, TRAF5 also bound the cytoplasmic domain of RANK.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Immunex Corporation
- (ii) TITLE OF INVENTION: Receptor Activator of NF-kappaB
- (iii) NUMBER OF SEQUENCES: 19
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Immunex Corporation, Law Department
 - (B) STREET: 51 University Street
 - (C) CITY: Seattle
 - (D) STATE: WA
 - (E) COUNTRY: USA
 - (F) ZIP: 98101
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: Apple Power Macintosh
 - (C) OPERATING SYSTEM: Apple Operating System 7.5.5
 - (D) SOFTWARE: Microsoft Word for Power Macintosh 6.0.1
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE: 22 DECEMBER 1997
 - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: USSN 60/064,671
 - (B) FILING DATE: 14 OCTOBER 1997
 - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: USSN 08/813,509
 - (B) FILING DATE: 07 MARCH 1997
 - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: USSN 08/772,330 (60/064,671)
 - (B) FILING DATE: 23 DECEMBER 1996
 - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Perkins, Patricia Anne
 - (B) REGISTRATION NUMBER: 34,693
 - (C) REFERENCE/DOCKET NUMBER: 2851-WO
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: (206)587-0430
 - (B) TELEFAX: (206)233-0644

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 3115 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single

- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: HOMO SAPIENS
- (vii) IMMEDIATE SOURCE:
 (A) LIBRARY: BONE-MARROW DERIVED DENDRITIC CELLS
 (B) CLONE: 9D-8A
- (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 93..1868

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GCTGCTGCTG CTCTGCGCGC TGCTCGCCCG GCTGCAGTTT TATCCAGAAA GAGCTGTGTG	60
GACTCTCTGC CTGACCTCAG TGTTCCTTTTC AG GTG GCT TTG CAG ATC GCT CCT	113
Val Ala Leu Gln Ile Ala Pro	
1 5	
CCA TGT ACC AGT GAG AAG CAT TAT GAG CAT CTG GGA CGG TGC TGT AAC	161
Pro Cys Thr Ser Glu Lys His Tyr Glu His Leu Gly Arg Cys Cys Asn	
10 15 20	
AAA TGT GAA CCA GGA AAG TAC ATG TCT TCT AAA TGC ACT ACT ACC TCT	209
Lys Cys Glu Pro Gly Lys Tyr Met Ser Ser Lys Cys Thr Thr Thr Ser	
25 30 35	
GAC AGT GTA TGT CTG CCC TGT GGC CCG GAT GAA TAC TTG GAT AGC TGG	257
Asp Ser Val Cys Leu Pro Cys Gly Pro Asp Glu Tyr Leu Asp Ser Trp	
40 45 50 55	
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Asn Glu Glu Asp Lys Cys Leu Leu His Lys Val Cys Asp Thr Gly Lys	
60 65 70	
GCC CTG GTG GCC GTG GTC GCC GGC AAC AGC ACG ACC CCC CGG CGC TGC	353
Ala Leu Val Ala Val Val Ala Gly Asn Ser Thr Thr Pro Arg Arg Cys	
75 80 85	
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90 95 100	
CGC AAC ACC GAG TGC GCG CCG GGC CTG GGC GCC CAG CAC CCG TTG CAG	449
Arg Asn Thr Glu Cys Ala Pro Gly Leu Gly Ala Gln His Pro Leu Gln	
105 110 115	
CTC AAC AAG GAC ACA GTG TGC AAA CCT TGC CTT GCA GGC TAC TTC TCT	497
Leu Asn Lys Asp Thr Val Cys Lys Pro Cys Leu Ala Gly Tyr Phe Ser	
120 125 130 135	

GAT	GCC	TTT	TCC	TCC	ACG	GAC	AAA	TGC	AGA	CCC	TGG	ACC	AAC	TGT	ACC	545
Asp	Ala	Phe	Ser	Ser	Thr	Asp	Lys	Cys	Arg	Pro	Trp	Thr	Asn	Cys	Thr	
			140						145					150		
TTC	CTT	GGA	AAG	AGA	GTA	GAA	CAT	CAT	GGG	ACA	GAG	AAA	TCC	GAT	GCG	593
Phe	Leu	Gly	Lys	Arg	Val	Glu	His	His	Gly	Thr	Glu	Lys	Ser	Asp	Ala	
			155					160					165			
GTT	TGC	AGT	TCT	TCT	CTG	CCA	GCT	AGA	AAA	CCA	CCA	AAT	GAA	CCC	CAT	641
Val	Cys	Ser	Ser	Ser	Leu	Pro	Ala	Arg	Lys	Pro	Pro	Asn	Glu	Pro	His	
		170					175					180				
GTT	TAC	TTG	CCC	GGT	TTA	ATA	ATT	CTG	CTT	CTC	TTC	GCG	TCT	GTG	GCC	689
Val	Tyr	Leu	Pro	Gly	Leu	Ile	Ile	Leu	Leu	Leu	Phe	Ala	Ser	Val	Ala	
	185					190					195					
CTG	GTG	GCT	GCC	ATC	ATC	TTT	GGC	GTT	TGC	TAT	AGG	AAA	AAA	GGG	AAA	737
Leu	Val	Ala	Ala	Ile	Ile	Phe	Gly	Val	Cys	Tyr	Arg	Lys	Lys	Gly	Lys	
200					205					210					215	
GCA	CTC	ACA	GCT	AAT	TTG	TGG	CAC	TGG	ATC	AAT	GAG	GCT	TGT	GGC	CGC	785
Ala	Leu	Thr	Ala	Asn	Leu	Trp	His	Trp	Ile	Asn	Glu	Ala	Cys	Gly	Arg	
				220					225					230		
CTA	AGT	GGA	GAT	AAG	GAG	TCC	TCA	GGT	GAC	AGT	TGT	GTC	AGT	ACA	CAC	833
Leu	Ser	Gly	Asp	Lys	Glu	Ser	Ser	Gly	Asp	Ser	Cys	Val	Ser	Thr	His	
			235					240					245			
ACG	GCA	AAC	TTT	GGT	CAG	CAG	GGA	GCA	TGT	GAA	GGT	GTC	TTA	CTG	CTG	881
Thr	Ala	Asn	Phe	Gly	Gln	Gln	Gly	Ala	Cys	Glu	Gly	Val	Leu	Leu	Leu	
		250					255					260				
ACT	CTG	GAG	GAG	AAG	ACA	TTT	CCA	GAA	GAT	ATG	TGC	TAC	CCA	GAT	CAA	929
Thr	Leu	Glu	Glu	Lys	Thr	Phe	Pro	Glu	Asp	Met	Cys	Tyr	Pro	Asp	Gln	
	265					270					275					
GGT	GGT	GTC	TGT	CAG	GGC	ACG	TGT	GTA	GGA	GGT	GGT	CCC	TAC	GCA	CAA	977
Gly	Gly	Val	Cys	Gln	Gly	Thr	Cys	Val	Gly	Gly	Gly	Pro	Tyr	Ala	Gln	
280					285					290					295	
GGC	GAA	GAT	GCC	AGG	ATG	CTC	TCA	TTG	GTC	AGC	AAG	ACC	GAG	ATA	GAG	1025
Gly	Glu	Asp	Ala	Arg	Met	Leu	Ser	Leu	Val	Ser	Lys	Thr	Glu	Ile	Glu	
				300					305					310		
GAA	GAC	AGC	TTC	AGA	CAG	ATG	CCC	ACA	GAA	GAT	GAA	TAC	ATG	GAC	AGG	1073
Glu	Asp	Ser	Phe	Arg	Gln	Met	Pro	Thr	Glu	Asp	Glu	Tyr	Met	Asp	Arg	
			315				320						325			
CCC	TCC	CAG	CCC	ACA	GAC	CAG	TTA	CTG	TTC	CTC	ACT	GAG	CCT	GGA	AGC	1121
Pro	Ser	Gln	Pro	Thr	Asp	Gln	Leu	Leu	Phe	Leu	Thr	Glu	Pro	Gly	Ser	
		330					335					340				
AAA	TCC	ACA	CCT	CCT	TTC	TCT	GAA	CCC	CTG	GAG	GTG	GGG	GAG	AAT	GAC	1169
Lys	Ser	Thr	Pro	Pro	Phe	Ser	Glu	Pro	Leu	Glu	Val	Gly	Glu	Asn	Asp	
	345					350					355					
AGT	TTA	AGC	CAG	TGC	TTC	ACG	GGG	ACA	CAG	AGC	ACA	GTG	GGT	TCA	GAA	1217
Ser	Leu	Ser	Gln	Cys	Phe	Thr	Gly	Thr	Gln	Ser	Thr	Val	Gly	Ser	Glu	
360					365					370					375	

AGC TGC AAC TGC ACT GAG CCC CTG TGC AGG ACT GAT TGG ACT CCC ATG	1265
Ser Cys Asn Cys Thr Glu Pro Leu Cys Arg Thr Asp Trp Thr Pro Met	
380 385 390	
TCC TCT GAA AAC TAC TTG CAA AAA GAG GTG GAC AGT GGC CAT TGC CCG	1313
Ser Ser Glu Asn Tyr Leu Gln Lys Glu Val Asp Ser Gly His Cys Pro	
395 400 405	
CAC TGG GCA GCC AGC CCC AGC CCC AAC TGG GCA GAT GTC TGC ACA GGC	1361
His Trp Ala Ala Ser Pro Ser Pro Asn Trp Ala Asp Val Cys Thr Gly	
410 415 420	
TGC CGG AAC CCT CCT GGG GAG GAC TGT GAA CCC CTC GTG GGT TCC CCA	1409
Cys Arg Asn Pro Pro Gly Glu Asp Cys Glu Pro Leu Val Gly Ser Pro	
425 430 435	
AAA CGT GGA CCC TTG CCC CAG TGC GCC TAT GGC ATG GGC CTT CCC CCT	1457
Lys Arg Gly Pro Leu Pro Gln Cys Ala Tyr Gly Met Gly Leu Pro Pro	
440 445 450 455	
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Glu Glu Glu Ala Ser Arg Thr Glu Ala Arg Asp Gln Pro Glu Asp Gly	
460 465 470	
GCT GAT GGG AGG CTC CCA AGC TCA GCG AGG GCA GGT GCC GGG TCT GGA	1553
Ala Asp Gly Arg Leu Pro Ser Ser Ala Arg Ala Gly Ala Gly Ser Gly	
475 480 485	
AGC TCC CCT GGT GGC CAG TCC CCT GCA TCT GGA AAT GTG ACT GGA AAC	1601
Ser Ser Pro Gly Gly Gln Ser Pro Ala Ser Gly Asn Val Thr Gly Asn	
490 495 500	
AGT AAC TCC ACG TTC ATC TCC AGC GGG CAG GTG ATG AAC TTC AAG GGC	1649
Ser Asn Ser Thr Phe Ile Ser Ser Gly Gln Val Met Asn Phe Lys Gly	
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520 525 530 535	
GCG GCT GCG GAG CCC ATG GGC CGC CCG GTG CAG GAG GAG ACC CTG GCG	1745
Ala Ala Ala Glu Pro Met Gly Arg Pro Val Gln Glu Glu Thr Leu Ala	
540 545 550	
CGC CGA GAC TCC TTC GCG GGG AAC GGC CCG CGC TTC CCG GAC CCG TGC	1793
Arg Arg Asp Ser Phe Ala Gly Asn Gly Pro Arg Phe Pro Asp Pro Cys	
555 560 565	
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Gly Gly Pro Glu Gly Leu Arg Glu Pro Glu Lys Ala Ser Arg Pro Val	
570 575 580	
CAG GAG CAA GGC GGG GCC AAG GCT TGA GCGCCCCCA TGGCTGGGAG	1888
Gln Glu Gln Gly Gly Ala Lys Ala	
585 590	
CCCGAAGCTC GGAGCCAGGG CTCGCGAGGG CAGCACCGCA GCCTCTGCCC CAGCCCCGGC	1948
CACCCAGGGA TCGATCGGTA CAGTCGAGGA AGACCACCCG GCATTCTCTG CCCACTTTGC	2008
CTTCCAGGAA ATGGGCTTTT CAGGAAGTGA ATTGATGAGG ACTGTCCCCA TGCCACGGA	2068

TGCTCAGCAG CCCGCCGCAC TGGGGCAGAT GTCTCCCCTG CCACTCCTCA AACTCGCAGC 2128
 AGTAATTTGT GGCACATATGA CAGCTATTTT TATGACTATC CTGTTCTGTG GGGGGGGGGT 2188
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 TGGTGCGATT ATAGCCCGGT GCAGCCTCTA ACTCCTGGGC TCAAGCAATC CAAGTGATCC 2428
 TCCCACCTCA ACCTTCGGAG TAGCTGGGAT CACAGCTGCA GGCCACGCCC AGCTTCCTCC 2488
 CCCCAGCTCC CCCCCCCCAG AGACACGGTC CCACCATGTT ACCCAGCCTG GTCTCAAACCT 2548
 CCCCAGCTAA AGCAGTCCTC CAGCCTCGGC CTCCCAAAGT ACTGGGATTA CAGGCGTGAG 2608
 CCCCACGCT GGCCTGCTTT ACGTATTTTC TTTTGTGCCC CTGCTCACAG TGTTTTAGAG 2668
 ATGGCTTTCC CAGTGTGTGT TCATTGTAAA CACTTTTGGG AAAGGGCTAA ACATGTGAGG 2728
 CCTGGAGATA GTTGCTAAGT TGCTAGGAAC ATGTGGTGGG ACTTTCATAT TCTGAAAAAT 2788
 GTTCTATATT CTCATTTTTC TAAAAGAAAG AAAAAAGGAA ACCCGATTTA TTTCTCCTGA 2848
 ATCTTTTTAA GTTTGTGTCG TTCCTTAAGC AGAACTAAGC TCAGTATGTG ACCTTACCCG 2908
 CTAGGTGGTT AATTTATCCA TGCTGGCAGA GGCACTCAGG TACTTGGTAA GCAAATTTCT 2968
 AAAACTCCAA GTTGCTGCAG CTTGGCATTC TTCTTATTCT AGAGGTCTCT CTGGAAAAGA 3028
 TGAGAAAAAT GAACAGGACA TGGGGCTCCT GGAAAGAAAG GGCCCGGGAA GTTCAAGGAA 3088
 GAATAAAGTT GAAATTTTAA AAAAAAA 3115

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 591 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Val	Ala	Leu	Gln	Ile	Ala	Pro	Pro	Cys	Thr	Ser	Glu	Lys	His	Tyr	Glu
1				5					10					15	
His	Leu	Gly	Arg	Cys	Cys	Asn	Lys	Cys	Glu	Pro	Gly	Lys	Tyr	Met	Ser
			20					25					30		
Ser	Lys	Cys	Thr	Thr	Thr	Ser	Asp	Ser	Val	Cys	Leu	Pro	Cys	Gly	Pro
			35				40					45			
Asp	Glu	Tyr	Leu	Asp	Ser	Trp	Asn	Glu	Glu	Asp	Lys	Cys	Leu	Leu	His
	50					55					60				

Lys	Val	Cys	Asp	Thr	Gly	Lys	Ala	Leu	Val	Ala	Val	Val	Ala	Gly	Asn	65	70	75	80
Ser	Thr	Thr	Pro	Arg	Arg	Cys	Ala	Cys	Thr	Ala	Gly	Tyr	His	Trp	Ser	85	90		95
Gln	Asp	Cys	Glu	Cys	Cys	Arg	Arg	Asn	Thr	Glu	Cys	Ala	Pro	Gly	Leu	100	105		110
Gly	Ala	Gln	His	Pro	Leu	Gln	Leu	Asn	Lys	Asp	Thr	Val	Cys	Lys	Pro	115	120		125
Cys	Leu	Ala	Gly	Tyr	Phe	Ser	Asp	Ala	Phe	Ser	Ser	Thr	Asp	Lys	Cys	130	135		140
Arg	Pro	Trp	Thr	Asn	Cys	Thr	Phe	Leu	Gly	Lys	Arg	Val	Glu	His	His	145	150	155	160
Gly	Thr	Glu	Lys	Ser	Asp	Ala	Val	Cys	Ser	Ser	Ser	Leu	Pro	Ala	Arg	165	170		175
Lys	Pro	Pro	Asn	Glu	Pro	His	Val	Tyr	Leu	Pro	Gly	Leu	Ile	Ile	Leu	180	185		190
Leu	Leu	Phe	Ala	Ser	Val	Ala	Leu	Val	Ala	Ala	Ile	Ile	Phe	Gly	Val	195	200		205
Cys	Tyr	Arg	Lys	Lys	Gly	Lys	Ala	Leu	Thr	Ala	Asn	Leu	Trp	His	Trp	210	215	220	
Ile	Asn	Glu	Ala	Cys	Gly	Arg	Leu	Ser	Gly	Asp	Lys	Glu	Ser	Ser	Gly	225	230	235	240
Asp	Ser	Cys	Val	Ser	Thr	His	Thr	Ala	Asn	Phe	Gly	Gln	Gln	Gly	Ala	245	250		255
Cys	Glu	Gly	Val	Leu	Leu	Leu	Thr	Leu	Glu	Glu	Lys	Thr	Phe	Pro	Glu	260	265		270
Asp	Met	Cys	Tyr	Pro	Asp	Gln	Gly	Gly	Val	Cys	Gln	Gly	Thr	Cys	Val	275	280	285	
Gly	Gly	Gly	Pro	Tyr	Ala	Gln	Gly	Glu	Asp	Ala	Arg	Met	Leu	Ser	Leu	290	295	300	
Val	Ser	Lys	Thr	Glu	Ile	Glu	Glu	Asp	Ser	Phe	Arg	Gln	Met	Pro	Thr	305	310	315	320
Glu	Asp	Glu	Tyr	Met	Asp	Arg	Pro	Ser	Gln	Pro	Thr	Asp	Gln	Leu	Leu	325	330		335
Phe	Leu	Thr	Glu	Pro	Gly	Ser	Lys	Ser	Thr	Pro	Pro	Phe	Ser	Glu	Pro	340	345		350
Leu	Glu	Val	Gly	Glu	Asn	Asp	Ser	Leu	Ser	Gln	Cys	Phe	Thr	Gly	Thr	355	360	365	
Gln	Ser	Thr	Val	Gly	Ser	Glu	Ser	Cys	Asn	Cys	Thr	Glu	Pro	Leu	Cys	370	375	380	

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Arg Thr Asp Trp Thr Pro Met Ser Ser Glu Asn Tyr Leu Gln Lys Glu
385                               390                               395                               400

Val Asp Ser Gly His Cys Pro His Trp Ala Ala Ser Pro Ser Pro Asn
                               405                               410                               415

Trp Ala Asp Val Cys Thr Gly Cys Arg Asn Pro Pro Gly Glu Asp Cys
                               420                               425                               430

Glu Pro Leu Val Gly Ser Pro Lys Arg Gly Pro Leu Pro Gln Cys Ala
                               435                               440                               445

Tyr Gly Met Gly Leu Pro Pro Glu Glu Glu Ala Ser Arg Thr Glu Ala
                               450                               455                               460

Arg Asp Gln Pro Glu Asp Gly Ala Asp Gly Arg Leu Pro Ser Ser Ala
465                               470                               475                               480

Arg Ala Gly Ala Gly Ser Gly Ser Ser Pro Gly Gly Gln Ser Pro Ala
                               485                               490                               495

Ser Gly Asn Val Thr Gly Asn Ser Asn Ser Thr Phe Ile Ser Ser Gly
                               500                               505                               510

Gln Val Met Asn Phe Lys Gly Asp Ile Ile Val Val Tyr Val Ser Gln
                               515                               520                               525

Thr Ser Gln Glu Gly Ala Ala Ala Ala Ala Glu Pro Met Gly Arg Pro
530                               535                               540

Val Gln Glu Glu Thr Leu Ala Arg Arg Asp Ser Phe Ala Gly Asn Gly
545                               550                               555                               560

Pro Arg Phe Pro Asp Pro Cys Gly Gly Pro Glu Gly Leu Arg Glu Pro
                               565                               570                               575

Glu Lys Ala Ser Arg Pro Val Gln Glu Gln Gly Gly Ala Lys Ala
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(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1391 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: HOMO SAPIENS
- (vii) IMMEDIATE SOURCE:
 - (A) LIBRARY: BONE-MARROW DERIVED DENDRITIC CELLS
 - (B) CLONE: 9D-15C

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 39..1391

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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                               Met Ala Pro Arg Ala
                               1           5

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Arg Arg Arg Arg Pro Leu Phe Ala Leu Leu Leu Leu Cys Ala Leu Leu
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GCC CGG CTG CAG GTG GCT TTG CAG ATC GCT CCT CCA TGT ACC AGT GAG      149
Ala Arg Leu Gln Val Ala Leu Gln Ile Ala Pro Pro Cys Thr Ser Glu
                25                30                35

AAG CAT TAT GAG CAT CTG GGA CGG TGC TGT AAC AAA TGT GAA CCA GGA      197
Lys His Tyr Glu His Leu Gly Arg Cys Cys Asn Lys Cys Glu Pro Gly
                40                45                50

AAG TAC ATG TCT TCT AAA TGC ACT ACT ACC TCT GAC AGT GTA TGT CTG      245
Lys Tyr Met Ser Ser Lys Cys Thr Thr Thr Ser Asp Ser Val Cys Leu
                55                60                65

CCC TGT GGC CCG GAT GAA TAC TTG GAT AGC TGG AAT GAA GAA GAT AAA      293
Pro Cys Gly Pro Asp Glu Tyr Leu Asp Ser Trp Asn Glu Glu Asp Lys
                70                75                80                85

TGC TTG CTG CAT AAA GTT TGT GAT ACA GGC AAG GCC CTG GTG GCC GTG      341
Cys Leu Leu His Lys Val Cys Asp Thr Gly Lys Ala Leu Val Ala Val
                90                95                100

GTC GCC GGC AAC AGC ACG ACC CCC CGG CGC TGC GCG TGC ACG GCT GGG      389
Val Ala Gly Asn Ser Thr Thr Pro Arg Arg Cys Ala Cys Thr Ala Gly
                105                110                115

TAC CAC TGG AGC CAG GAC TGC GAG TGC TGC CGC CGC AAC ACC GAG TGC      437
Tyr His Trp Ser Gln Asp Cys Glu Cys Cys Arg Arg Asn Thr Glu Cys
                120                125                130

GCG CCG GGC CTG GGC GCC CAG CAC CCG TTG CAG CTC AAC AAG GAC ACA      485
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                135                140                145

GTG TGC AAA CCT TGC CTT GCA GGC TAC TTC TCT GAT GCC TTT TCC TCC      533
Val Cys Lys Pro Cys Leu Ala Gly Tyr Phe Ser Asp Ala Phe Ser Ser
                150                155                160                165

ACG GAC AAA TGC AGA CCC TGG ACC AAC TGT ACC TTC CTT GGA AAG AGA      581
Thr Asp Lys Cys Arg Pro Trp Thr Asn Cys Thr Phe Leu Gly Lys Arg
                170                175                180

GTA GAA CAT CAT GGG ACA GAG AAA TCC GAT GCG GTT TGC AGT TCT TCT      629
Val Glu His His Gly Thr Glu Lys Ser Asp Ala Val Cys Ser Ser Ser
                185                190                195

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		200					205					210				
TTA	ATA	ATT	CTG	CTT	CTC	TTC	GCG	TCT	GTG	GCC	CTG	GTG	GCT	GCC	ATC	725
Leu	Ile	Ile	Leu	Leu	Leu	Phe	Ala	Ser	Val	Ala	Leu	Val	Ala	Ala	Ile	
	215					220					225					
ATC	TTT	GGC	GTT	TGC	TAT	AGG	AAA	AAA	GGG	AAA	GCA	CTC	ACA	GCT	AAT	773
Ile	Phe	Gly	Val	Cys	Tyr	Arg	Lys	Lys	Gly	Lys	Ala	Leu	Thr	Ala	Asn	
230					235					240					245	
TTG	TGG	CAC	TGG	ATC	AAT	GAG	GCT	TGT	GGC	CGC	CTA	AGT	GGA	GAT	AAG	821
Leu	Trp	His	Trp	Ile	Asn	Glu	Ala	Cys	Gly	Arg	Leu	Ser	Gly	Asp	Lys	
				250					255					260		
GAG	TCC	TCA	GGT	GAC	AGT	TGT	GTC	AGT	ACA	CAC	ACG	GCA	AAC	TTT	GGT	869
Glu	Ser	Ser	Gly	Asp	Ser	Cys	Val	Ser	Thr	His	Thr	Ala	Asn	Phe	Gly	
			265					270					275			
CAG	CAG	GGA	GCA	TGT	GAA	GGT	GTC	TTA	CTG	CTG	ACT	CTG	GAG	GAG	AAG	917
Gln	Gln	Gly	Ala	Cys	Glu	Gly	Val	Leu	Leu	Leu	Thr	Leu	Glu	Glu	Lys	
		280					285					290				
ACA	TTT	CCA	GAA	GAT	ATG	TGC	TAC	CCA	GAT	CAA	GGT	GGT	GTC	TGT	CAG	965
Thr	Phe	Pro	Glu	Asp	Met	Cys	Tyr	Pro	Asp	Gln	Gly	Gly	Val	Cys	Gln	
	295					300					305					
GGC	ACG	TGT	GTA	GGA	GGT	GGT	CCC	TAC	GCA	CAA	GGC	GAA	GAT	GCC	AGG	1013
Gly	Thr	Cys	Val	Gly	Gly	Gly	Pro	Tyr	Ala	Gln	Gly	Glu	Asp	Ala	Arg	
310					315					320					325	
ATG	CTC	TCA	TTG	GTC	AGC	AAG	ACC	GAG	ATA	GAG	GAA	GAC	AGC	TTC	AGA	1061
Met	Leu	Ser	Leu	Val	Ser	Lys	Thr	Glu	Ile	Glu	Glu	Asp	Ser	Phe	Arg	
				330					335					340		
CAG	ATG	CCC	ACA	GAA	GAT	GAA	TAC	ATG	GAC	AGG	CCC	TCC	CAG	CCC	ACA	1109
Gln	Met	Pro	Thr	Glu	Asp	Glu	Tyr	Met	Asp	Arg	Pro	Ser	Gln	Pro	Thr	
			345					350					355			
GAC	CAG	TTA	CTG	TTC	CTC	ACT	GAG	CCT	GGA	AGC	AAA	TCC	ACA	CCT	CCT	1157
Asp	Gln	Leu	Leu	Phe	Leu	Thr	Glu	Pro	Gly	Ser	Lys	Ser	Thr	Pro	Pro	
		360					365					370				
TTC	TCT	GAA	CCC	CTG	GAG	GTG	GGG	GAG	AAT	GAC	AGT	TTA	AGC	CAG	TGC	1205
Phe	Ser	Glu	Pro	Leu	Glu	Val	Gly	Glu	Asn	Asp	Ser	Leu	Ser	Gln	Cys	
	375					380					385					
TTC	ACG	GGG	ACA	CAG	AGC	ACA	GTG	GGT	TCA	GAA	AGC	TGC	AAC	TGC	ACT	1253
Phe	Thr	Gly	Thr	Gln	Ser	Thr	Val	Gly	Ser	Glu	Ser	Cys	Asn	Cys	Thr	
390					395					400					405	
GAG	CCC	CTG	TGC	AGG	ACT	GAT	TGG	ACT	CCC	ATG	TCC	TCT	GAA	AAC	TAC	1301
Glu	Pro	Leu	Cys	Arg	Thr	Asp	Trp	Thr	Pro	Met	Ser	Ser	Glu	Asn	Tyr	
				410					415					420		
TTG	CAA	AAA	GAG	GTG	GAC	AGT	GGC	CAT	TGC	CCG	CAC	TGG	GCA	GCC	AGC	1349
Leu	Gln	Lys	Glu	Val	Asp	Ser	Gly	His	Cys	Pro	His	Trp	Ala	Ala	Ser	
			425					430					435			

CCC AGC CCC AAC TGG GCA GAT GTC TGC ACA GGC TGC CGG AAC 1391
 Pro Ser Pro Asn Trp Ala Asp Val Cys Thr Gly Cys Arg Asn
 440 445 450

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 451 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Ala Pro Arg Ala Arg Arg Arg Arg Pro Leu Phe Ala Leu Leu Leu
 1 5 10 15
 Leu Cys Ala Leu Leu Ala Arg Leu Gln Val Ala Leu Gln Ile Ala Pro
 20 25 30
 Pro Cys Thr Ser Glu Lys His Tyr Glu His Leu Gly Arg Cys Cys Asn
 35 40 45
 Lys Cys Glu Pro Gly Lys Tyr Met Ser Ser Lys Cys Thr Thr Thr Ser
 50 55 60
 Asp Ser Val Cys Leu Pro Cys Gly Pro Asp Glu Tyr Leu Asp Ser Trp
 65 70 75 80
 Asn Glu Glu Asp Lys Cys Leu Leu His Lys Val Cys Asp Thr Gly Lys
 85 90 95
 Ala Leu Val Ala Val Val Ala Gly Asn Ser Thr Thr Pro Arg Arg Cys
 100 105 110
 Ala Cys Thr Ala Gly Tyr His Trp Ser Gln Asp Cys Glu Cys Cys Arg
 115 120 125
 Arg Asn Thr Glu Cys Ala Pro Gly Leu Gly Ala Gln His Pro Leu Gln
 130 135 140
 Leu Asn Lys Asp Thr Val Cys Lys Pro Cys Leu Ala Gly Tyr Phe Ser
 145 150 155 160
 Asp Ala Phe Ser Ser Thr Asp Lys Cys Arg Pro Trp Thr Asn Cys Thr
 165 170 175
 Phe Leu Gly Lys Arg Val Glu His His Gly Thr Glu Lys Ser Asp Ala
 180 185 190
 Val Cys Ser Ser Ser Leu Pro Ala Arg Lys Pro Pro Asn Glu Pro His
 195 200 205
 Val Tyr Leu Pro Gly Leu Ile Ile Leu Leu Leu Phe Ala Ser Val Ala
 210 215 220
 Leu Val Ala Ala Ile Ile Phe Gly Val Cys Tyr Arg Lys Lys Gly Lys
 225 230 235 240

(2) INFORMATION FOR SEQ ID NO:5:

(A) LENGTH: 3136 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: BONE-MARROW DERIVED DENDRITIC CELLS
 (B) CLONE: FULL LENGTH RANK

(ix) FEATURE:

(A) NAME/KEY: CDS
 (B) LOCATION: 39..1886

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CCGCTGAGGC CGCGGCGCCC GCCAGCCTGT CCCGCGCC ATG GCC CCG CGC GCC	53
Met Ala Pro Arg Ala	
1 5	
CGG CGG CGC CGC CCG CTG TTC GCG CTG CTG CTG CTC TGC GCG CTG CTC	101
Arg Arg Arg Arg Pro Leu Phe Ala Leu Leu Leu Leu Cys Ala Leu Leu	
10 15 20	
GCC CGG CTG CAG GTG GCT TTG CAG ATC GCT CCT CCA TGT ACC AGT GAG	149
Ala Arg Leu Gln Val Ala Leu Gln Ile Ala Pro Pro Cys Thr Ser Glu	
25 30 35	
AAG CAT TAT GAG CAT CTG GGA CGG TGC TGT AAC AAA TGT GAA CCA GGA	197
Lys His Tyr Glu His Leu Gly Arg Cys Cys Asn Lys Cys Glu Pro Gly	
40 45 50	
AAG TAC ATG TCT TCT AAA TGC ACT ACT ACC TCT GAC AGT GTA TGT CTG	245
Lys Tyr Met Ser Ser Lys Cys Thr Thr Thr Ser Asp Ser Val Cys Leu	
55 60 65	
CCC TGT GGC CCG GAT GAA TAC TTG GAT AGC TGG AAT GAA GAA GAT AAA	293
Pro Cys Gly Pro Asp Glu Tyr Leu Asp Ser Trp Asn Glu Glu Asp Lys	
70 75 80 85	
TGC TTG CTG CAT AAA GTT TGT GAT ACA GGC AAG GCC CTG GTG GCC GTG	341
Cys Leu Leu His Lys Val Cys Asp Thr Gly Lys Ala Leu Val Ala Val	
90 95 100	
GTC GCC GGC AAC AGC ACG ACC CCC CGG CGC TGC GCG TGC ACG GCT GGG	389
Val Ala Gly Asn Ser Thr Thr Pro Arg Cys Ala Cys Thr Ala Gly	
105 110 115	
TAC CAC TGG AGC CAG GAC TGC GAG TGC TGC CGC CGC AAC ACC GAG TGC	437
Tyr His Trp Ser Gln Asp Cys Glu Cys Cys Arg Arg Asn Thr Glu Cys	
120 125 130	
GCG CCG GGC CTG GGC GCC CAG CAC CCG TTG CAG CTC AAC AAG GAC ACA	485
Ala Pro Gly Leu Gly Ala Gln His Pro Leu Gln Leu Asn Lys Asp Thr	
135 140 145	
GTG TGC AAA CCT TGC CTT GCA GGC TAC TTC TCT GAT GCC TTT TCC TCC	533
Val Cys Lys Pro Cys Leu Ala Gly Tyr Phe Ser Asp Ala Phe Ser Ser	
150 155 160 165	
ACG GAC AAA TGC AGA CCC TGG ACC AAC TGT ACC TTC CTT GGA AAG AGA	581
Thr Asp Lys Cys Arg Pro Trp Thr Asn Cys Thr Phe Leu Gly Lys Arg	
170 175 180	
GTA GAA CAT CAT GGG ACA GAG AAA TCC GAT GCG GTT TGC AGT TCT TCT	629
Val Glu His His Gly Thr Glu Lys Ser Asp Ala Val Cys Ser Ser Ser	
185 190 195	

CTG	CCA	GCT	AGA	AAA	CCA	CCA	AAT	GAA	CCC	CAT	GTT	TAC	TTG	CCC	GGT	677
Leu	Pro	Ala	Arg	Lys	Pro	Pro	Asn	Glu	Pro	His	Val	Tyr	Leu	Pro	Gly	
	200						205					210				
TTA	ATA	ATT	CTG	CTT	CTC	TTC	GCG	TCT	GTG	GCC	CTG	GTG	GCT	GCC	ATC	725
Leu	Ile	Ile	Leu	Leu	Leu	Phe	Ala	Ser	Val	Ala	Leu	Val	Ala	Ala	Ile	
	215					220					225					
ATC	TTT	GGC	GTT	TGC	TAT	AGG	AAA	AAA	GGG	AAA	GCA	CTC	ACA	GCT	AAT	773
Ile	Phe	Gly	Val	Cys	Tyr	Arg	Lys	Lys	Gly	Lys	Ala	Leu	Thr	Ala	Asn	
230					235					240					245	
TTG	TGG	CAC	TGG	ATC	AAT	GAG	GCT	TGT	GGC	CGC	CTA	AGT	GGA	GAT	AAG	821
Leu	Trp	His	Trp	Ile	Asn	Glu	Ala	Cys	Gly	Arg	Leu	Ser	Gly	Asp	Lys	
				250					255					260		
GAG	TCC	TCA	GGT	GAC	AGT	TGT	GTC	AGT	ACA	CAC	ACG	GCA	AAC	TTT	GGT	869
Glu	Ser	Ser	Gly	Asp	Ser	Cys	Val	Ser	Thr	His	Thr	Ala	Asn	Phe	Gly	
			265					270					275			
CAG	CAG	GGA	GCA	TGT	GAA	GGT	GTC	TTA	CTG	CTG	ACT	CTG	GAG	GAG	AAG	917
Gln	Gln	Gly	Ala	Cys	Glu	Gly	Val	Leu	Leu	Leu	Thr	Leu	Glu	Glu	Lys	
		280					285					290				
ACA	TTT	CCA	GAA	GAT	ATG	TGC	TAC	CCA	GAT	CAA	GGT	GGT	GTC	TGT	CAG	965
Thr	Phe	Pro	Glu	Asp	Met	Cys	Tyr	Pro	Asp	Gln	Gly	Gly	Val	Cys	Gln	
	295					300					305					
GGC	ACG	TGT	GTA	GGA	GGT	GGT	CCC	TAC	GCA	CAA	GGC	GAA	GAT	GCC	AGG	1013
Gly	Thr	Cys	Val	Gly	Gly	Gly	Pro	Tyr	Ala	Gln	Gly	Glu	Asp	Ala	Arg	
310					315					320					325	
ATG	CTC	TCA	TTG	GTC	AGC	AAG	ACC	GAG	ATA	GAG	GAA	GAC	AGC	TTC	AGA	1061
Met	Leu	Ser	Leu	Val	Ser	Lys	Thr	Glu	Ile	Glu	Glu	Asp	Ser	Phe	Arg	
				330					335					340		
CAG	ATG	CCC	ACA	GAA	GAT	GAA	TAC	ATG	GAC	AGG	CCC	TCC	CAG	CCC	ACA	1109
Gln	Met	Pro	Thr	Glu	Asp	Glu	Tyr	Met	Asp	Arg	Pro	Ser	Gln	Pro	Thr	
			345					350					355			
GAC	CAG	TTA	CTG	TTC	CTC	ACT	GAG	CCT	GGA	AGC	AAA	TCC	ACA	CCT	CCT	1157
Asp	Gln	Leu	Leu	Phe	Leu	Thr	Glu	Pro	Gly	Ser	Lys	Ser	Thr	Pro	Pro	
		360					365					370				
TTC	TCT	GAA	CCC	CTG	GAG	GTG	GGG	GAG	AAT	GAC	AGT	TTA	AGC	CAG	TGC	1205
Phe	Ser	Glu	Pro	Leu	Glu	Val	Gly	Glu	Asn	Asp	Ser	Leu	Ser	Gln	Cys	
	375					380					385					
TTC	ACG	GGG	ACA	CAG	AGC	ACA	GTG	GGT	TCA	GAA	AGC	TGC	AAC	TGC	ACT	1253
Phe	Thr	Gly	Thr	Gln	Ser	Thr	Val	Gly	Ser	Glu	Ser	Cys	Asn	Cys	Thr	
390					395					400					405	
GAG	CCC	CTG	TGC	AGG	ACT	GAT	TGG	ACT	CCC	ATG	TCC	TCT	GAA	AAC	TAC	1301
Glu	Pro	Leu	Cys	Arg	Thr	Asp	Trp	Thr	Pro	Met	Ser	Ser	Glu	Asn	Tyr	
				410					415					420		
TTG	CAA	AAA	GAG	GTG	GAC	AGT	GGC	CAT	TGC	CCG	CAC	TGG	GCA	GCC	AGC	1349
Leu	Gln	Lys	Glu	Val	Asp	Ser	Gly	His	Cys	Pro	His	Trp	Ala	Ala	Ser	
			425					430					435			

CCC AGC CCC AAC TGG GCA GAT GTC TGC ACA GGC TGC CGG AAC CCT CCT	1397
Pro Ser Pro Asn Trp Ala Asp Val Cys Thr Gly Cys Arg Asn Pro Pro	
440 445 450	
GGG GAG GAC TGT GAA CCC CTC GTG GGT TCC CCA AAA CGT GGA CCC TTG	1445
Gly Glu Asp Cys Glu Pro Leu Val Gly Ser Pro Lys Arg Gly Pro Leu	
455 460 465	
CCC CAG TGC GCC TAT GGC ATG GGC CTT CCC CCT GAA GAA GAA GCC AGC	1493
Pro Gln Cys Ala Tyr Gly Met Gly Leu Pro Pro Glu Glu Glu Ala Ser	
470 475 480 485	
AGG ACG GAG GCC AGA GAC CAG CCC GAG GAT GGG GCT GAT GGG AGG CTC	1541
Arg Thr Glu Ala Arg Asp Gln Pro Glu Asp Gly Ala Asp Gly Arg Leu	
490 495 500	
CCA AGC TCA GCG AGG GCA GGT GCC GGG TCT GGA AGC TCC CCT GGT GGC	1589
Pro Ser Ser Ala Arg Ala Gly Ala Gly Ser Gly Ser Ser Pro Gly Gly	
505 510 515	
CAG TCC CCT GCA TCT GGA AAT GTG ACT GGA AAC AGT AAC TCC ACG TTC	1637
Gln Ser Pro Ala Ser Gly Asn Val Thr Gly Asn Ser Asn Ser Thr Phe	
520 525 530	
ATC TCC AGC GGG CAG GTG ATG AAC TTC AAG GGC GAC ATC ATC GTG GTC	1685
Ile Ser Ser Gly Gln Val Met Asn Phe Lys Gly Asp Ile Ile Val Val	
535 540 545	
TAC GTC AGC CAG ACC TCG CAG GAG GGC GCG GCG GCG GCT GCG GAG CCC	1733
Tyr Val Ser Gln Thr Ser Gln Glu Gly Ala Ala Ala Ala Ala Glu Pro	
550 555 560 565	
ATG GGC CGC CCG GTG CAG GAG GAG ACC CTG GCG CGC CGA GAC TCC TTC	1781
Met Gly Arg Pro Val Gln Glu Glu Thr Leu Ala Arg Arg Asp Ser Phe	
570 575 580	
GCG GGG AAC GGC CCG CGC TTC CCG GAC CCG TGC GGC GGC CCC GAG GGG	1829
Ala Gly Asn Gly Pro Arg Phe Pro Asp Pro Cys Gly Gly Pro Glu Gly	
585 590 595	
CTG CGG GAG CCG GAG AAG GCC TCG AGG CCG GTG CAG GAG CAA GGC GGG	1877
Leu Arg Glu Pro Glu Lys Ala Ser Arg Pro Val Gln Glu Gln Gly Gly	
600 605 610	
GCC AAG GCT TGAGCGCCCC CCATGGCTGG GAGCCCGAAG CTCGGAGCCA	1926
Ala Lys Ala	
615	
GGGCTCGCGA GGGCAGCACC GCAGCCTCTG CCCCAGCCCC GGCCACCCAG GGATCGATCG	1986
GTACAGTCGA GGAAGACCAC CCGGCATTCT CTGCCCCTT TGCCTTCCAG GAAATGGGCT	2046
TTTCAGGAAG TGAATTGATG AGGACTGTCC CCATGCCCCAC GGATGCTCAG CAGCCCGCCG	2106
CACTGGGGCA GATGTCTCCC CTGCCACTCC TCAAACTCGC AGCAGTAATT TGTGGCACTA	2166
TGACAGCTAT TTTTATGACT ATCCTGTTCT GTGGGGGGGG GGTCTATGTT TTCCCCCAT	2226
ATTTGTATTC CTTTTCATAA CTTTTCTTGA TATCTTTCCT CCCTCTTTTT TAATGTAAAG	2286
GTTTTCTCAA AAATTCTCCT AAAGGTGAGG GTCTCTTCT TTTCTCTTTT CCTTTTTTTT	2346

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TTCTTTTTTTT GGCAACCTGG CTCTGGCCCA GGCTAGAGTG CAGTGGTGCG ATTATAGCCC 2406
GGTGCAGCCT CTAACCTCCTG GGCTCAAGCA ATCCAAGTGA TCCTCCCACC TCAACCTTCG 2466
GAGTAGCTGG GATCACAGCT GCAGGCCACG CCCAGCTTCC TCCCCCGAC TCCCCCCCCC 2526
CAGAGACACG GTCCCACCAT GTTACCCAGC CTGGTCTCAA ACTCCCCAGC TAAAGCAGTC 2586
CTCCAGCCTC GGCCTCCCAA AGTACTGGGA TTACAGGCGT GAGCCCCCAC GCTGGCCTGC 2646
TTTACGTATT TTCTTTTGTG CCCCTGCTCA CAGTGTTTTTA GAGATGGCTT TCCCAGTGTG 2706
TGTTCAATTGT AAACACTTTT GGGAAAGGGC TAAACATGTG AGGCCTGGAG ATAGTTGCTA 2766
AGTTGCTAGG AACATGTGGT GGGACTTTCA TATTCTGAAA AATGTTCTAT ATTCTCATTT 2826
TTCTAAAAGA AAGAAAAAAG GAAACCCGAT TTATTTCTCC TGAATCTTTT TAAGTTTGTG 2886
TCGTTCCCTTA AGCAGAACTA AGCTCAGTAT GTGACCTTAC CCGCTAGGTG GTTAATTTAT 2946
CCATGCTGGC AGAGGCACTC AGGTACTTGG TAAGCAAATT TCTAAAACTC CAAGTTGCTG 3006
CAGCTTGGCA TTCTTCTTAT TCTAGAGGTC TCTCTGGAAA AGATGGAGAA AATGAACAGG 3066
ACATGGGGCT CCTGGAAAGA AAGGGCCCGG GAAGTTCAAG GAAGAATAAA GTTGAAATTT 3126
TAAAAAATAA 3136

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(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 616 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```

Met Ala Pro Arg Ala Arg Arg Arg Arg Pro Leu Phe Ala Leu Leu Leu
 1             5             10             15
Leu Cys Ala Leu Leu Ala Arg Leu Gln Val Ala Leu Gln Ile Ala Pro
      20             25             30
Pro Cys Thr Ser Glu Lys His Tyr Glu His Leu Gly Arg Cys Cys Asn
      35             40             45
Lys Cys Glu Pro Gly Lys Tyr Met Ser Ser Lys Cys Thr Thr Thr Ser
      50             55             60
Asp Ser Val Cys Leu Pro Cys Gly Pro Asp Glu Tyr Leu Asp Ser Trp
      65             70             75             80
Asn Glu Glu Asp Lys Cys Leu Leu His Lys Val Cys Asp Thr Gly Lys
      85             90             95
Ala Leu Val Ala Val Val Ala Gly Asn Ser Thr Thr Pro Arg Arg Cys
      100             105             110

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Ala Cys Thr Ala Gly Tyr His Trp Ser Gln Asp Cys Glu Cys Cys Arg
 115 120 125
 Arg Asn Thr Glu Cys Ala Pro Gly Leu Gly Ala Gln His Pro Leu Gln
 130 135 140
 Leu Asn Lys Asp Thr Val Cys Lys Pro Cys Leu Ala Gly Tyr Phe Ser
 145 150 155 160
 Asp Ala Phe Ser Ser Thr Asp Lys Cys Arg Pro Trp Thr Asn Cys Thr
 165 170 175
 Phe Leu Gly Lys Arg Val Glu His His Gly Thr Glu Lys Ser Asp Ala
 180 185 190
 Val Cys Ser Ser Ser Leu Pro Ala Arg Lys Pro Pro Asn Glu Pro His
 195 200 205
 Val Tyr Leu Pro Gly Leu Ile Ile Leu Leu Leu Phe Ala Ser Val Ala
 210 215 220
 Leu Val Ala Ala Ile Ile Phe Gly Val Cys Tyr Arg Lys Lys Gly Lys
 225 230 235 240
 Ala Leu Thr Ala Asn Leu Trp His Trp Ile Asn Glu Ala Cys Gly Arg
 245 250 255
 Leu Ser Gly Asp Lys Glu Ser Ser Gly Asp Ser Cys Val Ser Thr His
 260 265 270
 Thr Ala Asn Phe Gly Gln Gln Gly Ala Cys Glu Gly Val Leu Leu Leu
 275 280 285
 Thr Leu Glu Glu Lys Thr Phe Pro Glu Asp Met Cys Tyr Pro Asp Gln
 290 295 300
 Gly Gly Val Cys Gln Gly Thr Cys Val Gly Gly Gly Pro Tyr Ala Gln
 305 310 315 320
 Gly Glu Asp Ala Arg Met Leu Ser Leu Val Ser Lys Thr Glu Ile Glu
 325 330 335
 Glu Asp Ser Phe Arg Gln Met Pro Thr Glu Asp Glu Tyr Met Asp Arg
 340 345 350
 Pro Ser Gln Pro Thr Asp Gln Leu Leu Phe Leu Thr Glu Pro Gly Ser
 355 360 365
 Lys Ser Thr Pro Pro Phe Ser Glu Pro Leu Glu Val Gly Glu Asn Asp
 370 375 380
 Ser Leu Ser Gln Cys Phe Thr Gly Thr Gln Ser Thr Val Gly Ser Glu
 385 390 395 400
 Ser Cys Asn Cys Thr Glu Pro Leu Cys Arg Thr Asp Trp Thr Pro Met
 405 410 415
 Ser Ser Glu Asn Tyr Leu Gln Lys Glu Val Asp Ser Gly His Cys Pro
 420 425 430

His Trp Ala Ala Ser Pro Ser Pro Asn Trp Ala Asp Val Cys Thr Gly
 435 440 445
 Cys Arg Asn Pro Pro Gly Glu Asp Cys Glu Pro Leu Val Gly Ser Pro
 450 455 460
 Lys Arg Gly Pro Leu Pro Gln Cys Ala Tyr Gly Met Gly Leu Pro Pro
 465 470 475 480
 Glu Glu Glu Ala Ser Arg Thr Glu Ala Arg Asp Gln Pro Glu Asp Gly
 485 490 495
 Ala Asp Gly Arg Leu Pro Ser Ser Ala Arg Ala Gly Ala Gly Ser Gly
 500 505 510
 Ser Ser Pro Gly Gly Gln Ser Pro Ala Ser Gly Asn Val Thr Gly Asn
 515 520 525
 Ser Asn Ser Thr Phe Ile Ser Ser Gly Gln Val Met Asn Phe Lys Gly
 530 535 540
 Asp Ile Ile Val Val Tyr Val Ser Gln Thr Ser Gln Glu Gly Ala Ala
 545 550 555 560
 Ala Ala Ala Glu Pro Met Gly Arg Pro Val Gln Glu Glu Thr Leu Ala
 565 570 575
 Arg Arg Asp Ser Phe Ala Gly Asn Gly Pro Arg Phe Pro Asp Pro Cys
 580 585 590
 Gly Gly Pro Glu Gly Leu Arg Glu Pro Glu Lys Ala Ser Arg Pro Val
 595 600 605
 Gln Glu Gln Gly Gly Ala Lys Ala
 610 615

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vii) IMMEDIATE SOURCE:

- (B) CLONE: FLAG® peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Asp Tyr Lys Asp Asp Asp Asp Lys
 1 5

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 232 amino acids
- (B) TYPE: amino acid

(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Human

(vii) IMMEDIATE SOURCE:
(B) CLONE: IgG1 Fc mutein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Glu	Pro	Arg	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	1	5	10	15
Pro	Glu	Ala	Glu	Gly	Ala	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	20	25	30	
Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	35	40	45	
Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	50	55	60	
Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	65	70	75	80
Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	85	90	95	
Asp	Trp	Leu	Asn	Gly	Lys	Asp	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	100	105	110	
Leu	Pro	Ala	Pro	Met	Gln	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	115	120	125	
Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	130	135	140	
Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Arg	145	150	155	160
His	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	165	170	175	
Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	180	185	190	
Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	195	200	205	
Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	210	215	220	
Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys									225	230		

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 31 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: CMV (R2780 Leader)

(ix) FEATURE:

- (D) OTHER INFORMATION: Met1-Arg28 is the actual leader peptide; Arg29 strengthens the furin cleavage site; nucleotides encoding Thr30 and Ser31 add a SpeI site.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Met	Ala	Arg	Arg	Leu	Trp	Ile	Leu	Ser	Leu	Leu	Ala	Val	Thr	Leu	Thr
1				5				10					15		
Val	Ala	Leu	Ala	Ala	Pro	Ser	Gln	Lys	Ser	Lys	Arg	Arg	Thr	Ser	
			20					25					30		

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1630 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Mus musculus

(vii) IMMEDIATE SOURCE:

- (A) LIBRARY:
- (B) CLONE: RANKL

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 3..884

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CC	GGC	GTC	CCA	CAC	GAG	GGT	CCG	CTG	CAC	CCC	GCG	CCT	TCT	GCA	CCG
Gly	Val	Pro	His	Glu	Gly	Pro	Leu	His	Pro	Ala	Pro	Ser	Ala	Pro	
1				5				10					15		

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GCT	CCG	GCG	CCG	CCA	CCC	GCC	GCC	TCC	CGC	TCC	ATG	TTC	CTG	GCC	CTC	95
Ala	Pro	Ala	Pro	Pro	Pro	Ala	Ala	Ser	Arg	Ser	Met	Phe	Leu	Ala	Leu	
				20					25					30		
CTG	GGG	CTG	GGA	CTG	GGC	CAG	GTG	GTC	TGC	AGC	ATC	GCT	CTG	TTC	CTG	143
Leu	Gly	Leu	Gly	Leu	Gly	Gln	Val	Val	Cys	Ser	Ile	Ala	Leu	Phe	Leu	
			35				40						45			
TAC	TTT	CGA	GCG	CAG	ATG	GAT	CCT	AAC	AGA	ATA	TCA	GAA	GAC	AGC	ACT	191
Tyr	Phe	Arg	Ala	Gln	Met	Asp	Pro	Asn	Arg	Ile	Ser	Glu	Asp	Ser	Thr	
		50					55					60				
CAC	TGC	TTT	TAT	AGA	ATC	CTG	AGA	CTC	CAT	GAA	AAC	GCA	GAT	TTG	CAG	239
His	Cys	Phe	Tyr	Arg	Ile	Leu	Arg	Leu	His	Glu	Asn	Ala	Asp	Leu	Gln	
	65					70					75					
GAC	TCG	ACT	CTG	GAG	AGT	GAA	GAC	ACA	CTA	CCT	GAC	TCC	TGC	AGG	AGG	287
Asp	Ser	Thr	Leu	Glu	Ser	Glu	Asp	Thr	Leu	Pro	Asp	Ser	Cys	Arg	Arg	
	80				85					90					95	
ATG	AAA	CAA	GCC	TTT	CAG	GGG	GCC	GTG	CAG	AAG	GAA	CTG	CAA	CAC	ATT	335
Met	Lys	Gln	Ala	Phe	Gln	Gly	Ala	Val	Gln	Lys	Glu	Leu	Gln	His	Ile	
			100					105						110		
GTG	GGG	CCA	CAG	CGC	TTC	TCA	GGA	GCT	CCA	GCT	ATG	ATG	GAA	GGC	TCA	383
Val	Gly	Pro	Gln	Arg	Phe	Ser	Gly	Ala	Pro	Ala	Met	Met	Glu	Gly	Ser	
			115					120					125			
TGG	TTG	GAT	GTG	GCC	CAG	CGA	GGC	AAG	CCT	GAG	GCC	CAG	CCA	TTT	GCA	431
Trp	Leu	Asp	Val	Ala	Gln	Arg	Gly	Lys	Pro	Glu	Ala	Gln	Pro	Phe	Ala	
		130					135					140				
CAC	CTC	ACC	ATC	AAT	GCT	GCC	AGC	ATC	CCA	TCG	GGT	TCC	CAT	AAA	GTC	479
His	Leu	Thr	Ile	Asn	Ala	Ala	Ser	Ile	Pro	Ser	Gly	Ser	His	Lys	Val	
	145					150					155					
ACT	CTG	TCC	TCT	TGG	TAC	CAC	GAT	CGA	GGC	TGG	GCC	AAG	ATC	TCT	AAC	527
Thr	Leu	Ser	Ser	Trp	Tyr	His	Asp	Arg	Gly	Trp	Ala	Lys	Ile	Ser	Asn	
	160				165					170					175	
ATG	ACG	TTA	AGC	AAC	GGA	AAA	CTA	AGG	GTT	AAC	CAA	GAT	GGC	TTC	TAT	575
Met	Thr	Leu	Ser	Asn	Gly	Lys	Leu	Arg	Val	Asn	Gln	Asp	Gly	Phe	Tyr	
				180				185						190		
TAC	CTG	TAC	GCC	AAC	ATT	TGC	TTT	CGG	CAT	CAT	GAA	ACA	TCG	GGA	AGC	623
Tyr	Leu	Tyr	Ala	Asn	Ile	Cys	Phe	Arg	His	His	Glu	Thr	Ser	Gly	Ser	
			195					200					205			
GTA	CCT	ACA	GAC	TAT	CTT	CAG	CTG	ATG	GTG	TAT	GTC	GTT	AAA	ACC	AGC	671
Val	Pro	Thr	Asp	Tyr	Leu	Gln	Leu	Met	Val	Tyr	Val	Val	Lys	Thr	Ser	
		210					215					220				
ATC	AAA	ATC	CCA	AGT	TCT	CAT	AAC	CTG	ATG	AAA	GGA	GGG	AGC	ACG	AAA	719
Ile	Lys	Ile	Pro	Ser	Ser	His	Asn	Leu	Met	Lys	Gly	Gly	Ser	Thr	Lys	
	225					230					235					
AAC	TGG	TCG	GGC	AAT	TCT	GAA	TTC	CAC	TTT	TAT	TCC	ATA	AAT	GTT	GGG	767
Asn	Trp	Ser	Gly	Asn	Ser	Glu	Phe	His	Phe	Tyr	Ser	Ile	Asn	Val	Gly	
	240				245					250					255	

GGA TTT TTC AAG CTC CGA GCT GGT GAA GAA ATT AGC ATT CAG GTG TCC 815
 Gly Phe Phe Lys Leu Arg Ala Gly Glu Glu Ile Ser Ile Gln Val Ser
 260 265 270

AAC CCT TCC CTG CTG GAT CCG GAT CAA GAT GCG ACG TAC TTT GGG GCT 863
 Asn Pro Ser Leu Leu Asp Pro Asp Gln Asp Ala Thr Tyr Phe Gly Ala
 275 280 285

TTC AAA GTT CAG GAC ATA GAC TGAGACTCAT TTCGTGGAAC ATTAGCATGG 914
 Phe Lys Val Gln Asp Ile Asp
 290

ATGTCCTAGA TGTTTGGAAA CTTCTTAAAA AATGGATGAT GTCTATACAT GTGTAAGACT 974
 ACTAAGAGAC ATGGCCACG GTGTATGAAA CTCACAGCCC TCTCTCTTGA GCCTGTACAG 1034
 GTTGTGTATA TGTAAGTCC ATAGGTGATG TTAGATTCAT GGTGATTACA CAACGGTTTT 1094
 ACAATTTTGT AATGATTTCC TAGAATTGAA CCAGATTGGG AGAGGTATTC CGATGCTTAT 1154
 GAAAAACTTA CACGTGAGCT ATGGAAGGGG GTCACAGTCT CTGGGTCTAA CCCCTGGACA 1214
 TGTGCCACTG AGAACCTTGA AATTAAGAGG ATGCCATGTC ATTGCAAAGA AATGATAGTG 1274
 TGAAGGGTTA AGTTCTTTTG AATTGTTACA TTGCGCTGGG ACCTGCAAAT AAGTTCTTTT 1334
 TTTCTAATGA GGAGAGAAAA ATATATGTAT TTTTATATAA TGTCTAAAGT TATATTTTCA 1394
 GTGTAATGTT TTCTGTGCAA AGTTTTGTAA ATTATATTTG TGCTATAGTA TTTGATTCAA 1454
 AATATTTAAA AATGTCTCAC TGTTGACATA TTTAATGTTT TAAATGTACA GATGTATTTA 1514
 ACTGGTGCAC TTTGTAATTC CCCTGAAGGT ACTCGTAGCT AAGGGGGCAG AATACTGTTT 1574
 CTGGTGACCA CATGTAGTTT ATTTCTTTAT TCTTTTAAAC TTAATAGAGT CTTTCA 1630

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 294 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Gly Val Pro His Glu Gly Pro Leu His Pro Ala Pro Ser Ala Pro Ala
 1 5 10 15

Pro Ala Pro Pro Pro Ala Ala Ser Arg Ser Met Phe Leu Ala Leu Leu
 20 25 30

Gly Leu Gly Leu Gly Gln Val Val Cys Ser Ile Ala Leu Phe Leu Tyr
 35 40 45

Phe Arg Ala Gln Met Asp Pro Asn Arg Ile Ser Glu Asp Ser Thr His
 50 55 60

Cys Phe Tyr Arg Ile Leu Arg Leu His Glu Asn Ala Asp Leu Gln Asp
 65 70 75 80
 Ser Thr Leu Glu Ser Glu Asp Thr Leu Pro Asp Ser Cys Arg Arg Met
 85 90 95
 Lys Gln Ala Phe Gln Gly Ala Val Gln Lys Glu Leu Gln His Ile Val
 100 105 110
 Gly Pro Gln Arg Phe Ser Gly Ala Pro Ala Met Met Glu Gly Ser Trp
 115 120 125
 Leu Asp Val Ala Gln Arg Gly Lys Pro Glu Ala Gln Pro Phe Ala His
 130 135 140
 Leu Thr Ile Asn Ala Ala Ser Ile Pro Ser Gly Ser His Lys Val Thr
 145 150 155 160
 Leu Ser Ser Trp Tyr His Asp Arg Gly Trp Ala Lys Ile Ser Asn Met
 165 170 175
 Thr Leu Ser Asn Gly Lys Leu Arg Val Asn Gln Asp Gly Phe Tyr Tyr
 180 185 190
 Leu Tyr Ala Asn Ile Cys Phe Arg His His Glu Thr Ser Gly Ser Val
 195 200 205
 Pro Thr Asp Tyr Leu Gln Leu Met Val Tyr Val Val Lys Thr Ser Ile
 210 215 220
 Lys Ile Pro Ser Ser His Asn Leu Met Lys Gly Gly Ser Thr Lys Asn
 225 230 235 240
 Trp Ser Gly Asn Ser Glu Phe His Phe Tyr Ser Ile Asn Val Gly Gly
 245 250 255
 Phe Phe Lys Leu Arg Ala Gly Glu Glu Ile Ser Ile Gln Val Ser Asn
 260 265 270
 Pro Ser Leu Leu Asp Pro Asp Gln Asp Ala Thr Tyr Phe Gly Ala Phe
 275 280 285
 Lys Val Gln Asp Ile Asp
 290

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 954 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(vii) IMMEDIATE SOURCE:

(A) LIBRARY:

(B) CLONE: huRANKL (full length)

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..951

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

ATG	CGC	CGC	GCC	AGC	AGA	GAC	TAC	ACC	AAG	TAC	CTG	CGT	GGC	TCG	GAG	48
Met	Arg	Arg	Ala	Ser	Arg	Asp	Tyr	Thr	Lys	Tyr	Leu	Arg	Gly	Ser	Glu	
1				5					10					15		
GAG	ATG	GGC	GGC	GGC	CCC	GGA	GCC	CCG	CAC	GAG	GGC	CCC	CTG	CAC	GCC	96
Glu	Met	Gly	Gly	Gly	Pro	Gly	Ala	Pro	His	Glu	Gly	Pro	Leu	His	Ala	
			20					25					30			
CCG	CCG	CCG	CCT	GCG	CCG	CAC	CAG	CCC	CCC	GCC	GCC	TCC	CGC	TCC	ATG	144
Pro	Pro	Pro	Pro	Ala	Pro	His	Gln	Pro	Pro	Ala	Ala	Ser	Arg	Ser	Met	
			35				40					45				
TTC	GTG	GCC	CTC	CTG	GGG	CTG	GGG	CTG	GGC	CAG	GTT	GTC	TGC	AGC	GTC	192
Phe	Val	Ala	Leu	Leu	Gly	Leu	Gly	Leu	Gly	Gln	Val	Val	Cys	Ser	Val	
	50					55					60					
GCC	CTG	TTC	TTC	TAT	TTC	AGA	GCG	CAG	ATG	GAT	CCT	AAT	AGA	ATA	TCA	240
Ala	Leu	Phe	Phe	Tyr	Phe	Arg	Ala	Gln	Met	Asp	Pro	Asn	Arg	Ile	Ser	
65					70				75						80	
GAA	GAT	GGC	ACT	CAC	TGC	ATT	TAT	AGA	ATT	TTG	AGA	CTC	CAT	GAA	AAT	288
Glu	Asp	Gly	Thr	His	Cys	Ile	Tyr	Arg	Ile	Leu	Arg	Leu	His	Glu	Asn	
				85					90					95		
GCA	GAT	TTT	CAA	GAC	ACA	ACT	CTG	GAG	AGT	CAA	GAT	ACA	AAA	TTA	ATA	336
Ala	Asp	Phe	Gln	Asp	Thr	Thr	Leu	Glu	Ser	Gln	Asp	Thr	Lys	Leu	Ile	
			100					105					110			
CCT	GAT	TCA	TGT	AGG	AGA	ATT	AAA	CAG	GCC	TTT	CAA	GGA	GCT	GTG	CAA	384
Pro	Asp	Ser	Cys	Arg	Arg	Ile	Lys	Gln	Ala	Phe	Gln	Gly	Ala	Val	Gln	
			115				120					125				
AAG	GAA	TTA	CAA	CAT	ATC	GTT	GGA	TCA	CAG	CAC	ATC	AGA	GCA	GAG	AAA	432
Lys	Glu	Leu	Gln	His	Ile	Val	Gly	Ser	Gln	His	Ile	Arg	Ala	Glu	Lys	
	130					135					140					
GCG	ATG	GTG	GAT	GGC	TCA	TGG	TTA	GAT	CTG	GCC	AAG	AGG	AGC	AAG	CTT	480
Ala	Met	Val	Asp	Gly	Ser	Trp	Leu	Asp	Leu	Ala	Lys	Arg	Ser	Lys	Leu	
145					150					155					160	
GAA	GCT	CAG	CCT	TTT	GCT	CAT	CTC	ACT	ATT	AAT	GCC	ACC	GAC	ATC	CCA	528
Glu	Ala	Gln	Pro	Phe	Ala	His	Leu	Thr	Ile	Asn	Ala	Thr	Asp	Ile	Pro	
				165					170					175		
TCT	GGT	TCC	CAT	AAA	GTG	AGT	CTG	TCC	TCT	TGG	TAC	CAT	GAT	CGG	GGT	576
Ser	Gly	Ser	His	Lys	Val	Ser	Leu	Ser	Ser	Trp	Tyr	His	Asp	Arg	Gly	
			180					185						190		

TGG	GCC	AAG	ATC	TCC	AAC	ATG	ACT	TTT	AGC	AAT	GGA	AAA	CTA	ATA	GTT	624
Trp	Ala	Lys	Ile	Ser	Asn	Met	Thr	Phe	Ser	Asn	Gly	Lys	Leu	Ile	Val	
		195					200					205				
AAT	CAG	GAT	GGC	TTT	TAT	TAC	CTG	TAT	GCC	AAC	ATT	TGC	TTT	CGA	CAT	672
Asn	Gln	Asp	Gly	Phe	Tyr	Tyr	Leu	Tyr	Ala	Asn	Ile	Cys	Phe	Arg	His	
	210					215					220					
CAT	GAA	ACT	TCA	GGA	GAC	CTA	GCT	ACA	GAG	TAT	CTT	CAA	CTA	ATG	GTG	720
His	Glu	Thr	Ser	Gly	Asp	Leu	Ala	Thr	Glu	Tyr	Leu	Gln	Leu	Met	Val	
	225				230					235					240	
TAC	GTC	ACT	AAA	ACC	AGC	ATC	AAA	ATC	CCA	AGT	TCT	CAT	ACC	CTG	ATG	768
Tyr	Val	Thr	Lys	Thr	Ser	Ile	Lys	Ile	Pro	Ser	Ser	His	Thr	Leu	Met	
				245					250					255		
AAA	GGA	GGA	AGC	ACC	AAG	TAT	TGG	TCA	GGG	AAT	TCT	GAA	TTC	CAT	TTT	816
Lys	Gly	Gly	Ser	Thr	Lys	Tyr	Trp	Ser	Gly	Asn	Ser	Glu	Phe	His	Phe	
			260					265					270			
TAT	TCC	ATA	AAC	GTT	GGT	GGA	TTT	TTT	AAG	TTA	CGG	TCT	GGA	GAG	GAA	864
Tyr	Ser	Ile	Asn	Val	Gly	Gly	Phe	Phe	Lys	Leu	Arg	Ser	Gly	Glu	Glu	
		275					280					285				
ATC	AGC	ATC	GAG	GTC	TCC	AAC	CCC	TCC	TTA	CTG	GAT	CCG	GAT	CAG	GAT	912
Ile	Ser	Ile	Glu	Val	Ser	Asn	Pro	Ser	Leu	Leu	Asp	Pro	Asp	Gln	Asp	
	290					295					300					
GCA	ACA	TAC	TTT	GGG	GCT	TTT	AAA	GTT	CGA	GAT	ATA	GAT	TGA			954
Ala	Thr	Tyr	Phe	Gly	Ala	Phe	Lys	Val	Arg	Asp	Ile	Asp				
	305				310					315						

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 317 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Met	Arg	Arg	Ala	Ser	Arg	Asp	Tyr	Thr	Lys	Tyr	Leu	Arg	Gly	Ser	Glu
1				5					10					15	
Glu	Met	Gly	Gly	Gly	Pro	Gly	Ala	Pro	His	Glu	Gly	Pro	Leu	His	Ala
		20						25					30		
Pro	Pro	Pro	Pro	Ala	Pro	His	Gln	Pro	Pro	Ala	Ala	Ser	Arg	Ser	Met
		35					40					45			
Phe	Val	Ala	Leu	Leu	Gly	Leu	Gly	Leu	Gly	Gln	Val	Val	Cys	Ser	Val
	50					55				60					
Ala	Leu	Phe	Phe	Tyr	Phe	Arg	Ala	Gln	Met	Asp	Pro	Asn	Arg	Ile	Ser
	65				70					75					80
Glu	Asp	Gly	Thr	His	Cys	Ile	Tyr	Arg	Ile	Leu	Arg	Leu	His	Glu	Asn
				85					90					95	

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Ala Asp Phe Gln Asp Thr Thr Leu Glu Ser Gln Asp Thr Lys Leu Ile
      100                      105                      110
Pro Asp Ser Cys Arg Arg Ile Lys Gln Ala Phe Gln Gly Ala Val Gln
      115                      120                      125
Lys Glu Leu Gln His Ile Val Gly Ser Gln His Ile Arg Ala Glu Lys
      130                      135                      140
Ala Met Val Asp Gly Ser Trp Leu Asp Leu Ala Lys Arg Ser Lys Leu
      145                      150                      155                      160
Glu Ala Gln Pro Phe Ala His Leu Thr Ile Asn Ala Thr Asp Ile Pro
      165                      170                      175
Ser Gly Ser His Lys Val Ser Leu Ser Ser Trp Tyr His Asp Arg Gly
      180                      185                      190
Trp Ala Lys Ile Ser Asn Met Thr Phe Ser Asn Gly Lys Leu Ile Val
      195                      200                      205
Asn Gln Asp Gly Phe Tyr Tyr Leu Tyr Ala Asn Ile Cys Phe Arg His
      210                      215                      220
His Glu Thr Ser Gly Asp Leu Ala Thr Glu Tyr Leu Gln Leu Met Val
      225                      230                      235                      240
Tyr Val Thr Lys Thr Ser Ile Lys Ile Pro Ser Ser His Thr Leu Met
      245                      250                      255
Lys Gly Gly Ser Thr Lys Tyr Trp Ser Gly Asn Ser Glu Phe His Phe
      260                      265                      270
Tyr Ser Ile Asn Val Gly Gly Phe Phe Lys Leu Arg Ser Gly Glu Glu
      275                      280                      285
Ile Ser Ile Glu Val Ser Asn Pro Ser Leu Leu Asp Pro Asp Gln Asp
      290                      295                      300
Ala Thr Tyr Phe Gly Ala Phe Lys Val Arg Asp Ile Asp
      305                      310                      315

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(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1878 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Murine

(vii) IMMEDIATE SOURCE:

- (A) LIBRARY: Murine Fetal Liver Epithelium
(B) CLONE: muRANK

(ix) FEATURE:

- (A) NAME/KEY: CDS
(B) LOCATION: 1..1875

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

ATG	GCC	CCG	CGC	GCC	CGG	CGG	CGC	CGC	CAG	CTG	CCC	GCG	CCG	CTG	CTG	48
Met	Ala	Pro	Arg	Ala	Arg	Arg	Arg	Arg	Gln	Leu	Pro	Ala	Pro	Leu	Leu	
1				5					10					15		
GCG	CTC	TGC	GTG	CTG	CTC	GTT	CCA	CTG	CAG	GTG	ACT	CTC	CAG	GTC	ACT	96
Ala	Leu	Cys	Val	Leu	Leu	Val	Pro	Leu	Gln	Val	Thr	Leu	Gln	Val	Thr	
			20					25					30			
CCT	CCA	TGC	ACC	CAG	GAG	AGG	CAT	TAT	GAG	CAT	CTC	GGA	CGG	TGT	TGC	144
Pro	Pro	Cys	Thr	Gln	Glu	Arg	His	Tyr	Glu	His	Leu	Gly	Arg	Cys	Cys	
		35					40					45				
AGC	AGA	TGC	GAA	CCA	GGA	AAG	TAC	CTG	TCC	TCT	AAG	TGC	ACT	CCT	ACC	192
Ser	Arg	Cys	Glu	Pro	Gly	Lys	Tyr	Leu	Ser	Ser	Lys	Cys	Thr	Pro	Thr	
	50					55					60					
TCC	GAC	AGT	GTG	TGT	CTG	CCC	TGT	GGC	CCC	GAT	GAG	TAC	TTG	GAC	ACC	240
Ser	Asp	Ser	Val	Cys	Leu	Pro	Cys	Gly	Pro	Asp	Glu	Tyr	Leu	Asp	Thr	
65					70				75					80		
TGG	AAT	GAA	GAA	GAT	AAA	TGC	TTG	CTG	CAT	AAA	GTC	TGT	GAT	GCA	GGC	288
Trp	Asn	Glu	Glu	Asp	Lys	Cys	Leu	Leu	His	Lys	Val	Cys	Asp	Ala	Gly	
			85					90						95		
AAG	GCC	CTG	GTG	GCG	GTG	GAT	CCT	GGC	AAC	CAC	ACG	GCC	CCG	CGT	CGC	336
Lys	Ala	Leu	Val	Ala	Val	Asp	Pro	Gly	Asn	His	Thr	Ala	Pro	Arg	Arg	
		100					105					110				
TGT	GCT	TGC	ACG	GCT	GGC	TAC	CAC	TGG	AAC	TCA	GAC	TGC	GAG	TGC	TGC	384
Cys	Ala	Cys	Thr	Ala	Gly	Tyr	His	Trp	Asn	Ser	Asp	Cys	Glu	Cys	Cys	
		115				120						125				
CGC	AGG	AAC	ACG	GAG	TGT	GCA	CCT	GGC	TTC	GGA	GCT	CAG	CAT	CCC	TTG	432
Arg	Arg	Asn	Thr	Glu	Cys	Ala	Pro	Gly	Phe	Gly	Ala	Gln	His	Pro	Leu	
	130					135				140						
CAG	CTC	AAC	AAG	GAT	ACG	GTG	TGC	ACA	CCC	TGC	CTC	CTG	GGC	TTC	TTC	480
Gln	Leu	Asn	Lys	Asp	Thr	Val	Cys	Thr	Pro	Cys	Leu	Leu	Gly	Phe	Phe	
145					150				155					160		
TCA	GAT	GTC	TTT	TCG	TCC	ACA	GAC	AAA	TGC	AAA	CCT	TGG	ACC	AAC	TGC	528
Ser	Asp	Val	Phe	Ser	Ser	Thr	Asp	Lys	Cys	Lys	Pro	Trp	Thr	Asn	Cys	
			165					170						175		
ACC	CTC	CTT	GGA	AAG	CTA	GAA	GCA	CAC	CAG	GGG	ACA	ACG	GAA	TCA	GAT	576
Thr	Leu	Leu	Gly	Lys	Leu	Glu	Ala	His	Gln	Gly	Thr	Thr	Glu	Ser	Asp	
		180						185					190			

GTG	GTC	TGC	AGC	TCT	TCC	ATG	ACA	CTG	AGG	AGA	CCA	CCC	AAG	GAG	GCC	624
Val	Val	Cys	Ser	Ser	Ser	Met	Thr	Leu	Arg	Arg	Pro	Pro	Lys	Glu	Ala	
		195					200					205				
CAG	GCT	TAC	CTG	CCC	AGT	CTC	ATC	GTT	CTG	CTC	CTC	TTC	ATC	TCT	GTG	672
Gln	Ala	Tyr	Leu	Pro	Ser	Leu	Ile	Val	Leu	Leu	Leu	Phe	Ile	Ser	Val	
	210					215					220					
GTA	GTA	GTG	GCT	GCC	ATC	ATC	TTC	GGC	GTT	TAC	TAC	AGG	AAG	GGA	GGG	720
Val	Val	Val	Ala	Ala	Ile	Ile	Phe	Gly	Val	Tyr	Tyr	Arg	Lys	Gly	Gly	
225					230					235					240	
AAA	GCG	CTG	ACA	GCT	AAT	TTG	TGG	AAT	TGG	GTC	AAT	GAT	GCT	TGC	AGT	768
Lys	Ala	Leu	Thr	Ala	Asn	Leu	Trp	Asn	Trp	Val	Asn	Asp	Ala	Cys	Ser	
			245					250						255		
AGT	CTA	AGT	GGA	AAT	AAG	GAG	TCC	TCA	GGG	GAC	CGT	TGT	GCT	GGT	TCC	816
Ser	Leu	Ser	Gly	Asn	Lys	Glu	Ser	Ser	Gly	Asp	Arg	Cys	Ala	Gly	Ser	
			260					265					270			
CAC	TCG	GCA	ACC	TCC	AGT	CAG	CAA	GAA	GTG	TGT	GAA	GGT	ATC	TTA	CTA	864
His	Ser	Ala	Thr	Ser	Ser	Gln	Gln	Glu	Val	Cys	Glu	Gly	Ile	Leu	Leu	
		275					280					285				
ATG	ACT	CGG	GAG	GAG	AAG	ATG	GTT	CCA	GAA	GAC	GGT	GCT	GGA	GTC	TGT	912
Met	Thr	Arg	Glu	Glu	Lys	Met	Val	Pro	Glu	Asp	Gly	Ala	Gly	Val	Cys	
	290					295					300					
GGG	CCT	GTG	TGT	GCG	GCA	GGT	GGG	CCC	TGG	GCA	GAA	GTC	AGA	GAT	TCT	960
Gly	Pro	Val	Cys	Ala	Ala	Gly	Gly	Pro	Trp	Ala	Glu	Val	Arg	Asp	Ser	
305					310					315					320	
AGG	ACG	TTC	ACA	CTG	GTC	AGC	GAG	GTT	GAG	ACG	CAA	GGA	GAC	CTC	TCG	1008
Arg	Thr	Phe	Thr	Leu	Val	Ser	Glu	Val	Glu	Thr	Gln	Gly	Asp	Leu	Ser	
				325					330					335		
AGG	AAG	ATT	CCC	ACA	GAG	GAT	GAG	TAC	ACG	GAC	CGG	CCC	TCG	CAG	CCT	1056
Arg	Lys	Ile	Pro	Thr	Glu	Asp	Glu	Tyr	Thr	Asp	Arg	Pro	Ser	Gln	Pro	
			340					345					350			
TCG	ACT	GGT	TCA	CTG	CTC	CTA	ATC	CAG	CAG	GGA	AGC	AAA	TCT	ATA	CCC	1104
Ser	Thr	Gly	Ser	Leu	Leu	Leu	Ile	Gln	Gln	Gly	Ser	Lys	Ser	Ile	Pro	
		355					360					365				
CCA	TTC	CAG	GAG	CCC	CTG	GAA	GTG	GGG	GAG	AAC	GAC	AGT	TTA	AGC	CAG	1152
Pro	Phe	Gln	Glu	Pro	Leu	Glu	Val	Gly	Glu	Asn	Asp	Ser	Leu	Ser	Gln	
	370					375					380					
TGT	TTC	ACC	GGG	ACT	GAA	AGC	ACG	GTG	GAT	TCT	GAG	GGC	TGT	GAC	TTC	1200
Cys	Phe	Thr	Gly	Thr	Glu	Ser	Thr	Val	Asp	Ser	Glu	Gly	Cys	Asp	Phe	
385					390					395					400	
ACT	GAG	CCT	CCG	AGC	AGA	ACT	GAC	TCT	ATG	CCC	GTG	TCC	CCT	GAA	AAG	1248
Thr	Glu	Pro	Pro	Ser	Arg	Thr	Asp	Ser	Met	Pro	Val	Ser	Pro	Glu	Lys	
				405					410					415		
CAC	CTG	ACA	AAA	GAA	ATA	GAA	GGT	GAC	AGT	TGC	CTC	CCC	TGG	GTG	GTC	1296
His	Leu	Thr	Lys	Glu	Ile	Glu	Gly	Asp	Ser	Cys	Leu	Pro	Trp	Val	Val	
			420					425					430			

AGC TCC AAC TCA ACA GAT GGC TAC ACA GGC AGT GGG AAC ACT CCT GGG	1344
Ser Ser Asn Ser Thr Asp Gly Tyr Thr Gly Ser Gly Asn Thr Pro Gly	
435 440 445	
GAG GAC CAT GAA CCC TTT CCA GGG TCC CTG AAA TGT GGA CCA TTG CCC	1392
Glu Asp His Glu Pro Phe Pro Gly Ser Leu Lys Cys Gly Pro Leu Pro	
450 455 460	
CAG TGT GCC TAC AGC ATG GGC TTT CCC AGT GAA GCA GCA GCC AGC ATG	1440
Gln Cys Ala Tyr Ser Met Gly Phe Pro Ser Glu Ala Ala Ala Ser Met	
465 470 475 480	
GCA GAG GCG GGA GTA CGG CCC CAG GAC AGG GCT GAT GAG AGG GGA GCC	1488
Ala Glu Ala Gly Val Arg Pro Gln Asp Arg Ala Asp Glu Arg Gly Ala	
485 490 495	
TCA GGG TCC GGG AGC TCC CCC AGT GAC CAG CCA CCT GCC TCT GGG AAC	1536
Ser Gly Ser Gly Ser Ser Pro Ser Asp Gln Pro Pro Ala Ser Gly Asn	
500 505 510	
GTG ACT GGA AAC AGT AAC TCC ACG TTC ATC TCT AGC GGG CAG GTG ATG	1584
Val Thr Gly Asn Ser Asn Ser Thr Phe Ile Ser Ser Gly Gln Val Met	
515 520 525	
AAC TTC AAG GGT GAC ATC ATC GTG GTG TAT GTC AGC CAG ACC TCG CAG	1632
Asn Phe Lys Gly Asp Ile Ile Val Val Tyr Val Ser Gln Thr Ser Gln	
530 535 540	
GAG GGC CCG GGT TCC GCA GAG CCC GAG TCG GAG CCC GTG GGC CGC CCT	1680
Glu Gly Pro Gly Ser Ala Glu Pro Glu Ser Glu Pro Val Gly Arg Pro	
545 550 555 560	
GTG CAG GAG GAG ACG CTG GCA CAC AGA GAC TCC TTT GCG GGC ACC GCG	1728
Val Gln Glu Glu Thr Leu Ala His Arg Asp Ser Phe Ala Gly Thr Ala	
565 570 575	
CCG CGC TTC CCC GAC GTC TGT GCC ACC GGG GCT GGG CTG CAG GAG CAG	1776
Pro Arg Phe Pro Asp Val Cys Ala Thr Gly Ala Gly Leu Gln Glu Gln	
580 585 590	
GGG GCA CCC CGG CAG AAG GAC GGG ACA TCG CGG CCG GTG CAG GAG CAG	1824
Gly Ala Pro Arg Gln Lys Asp Gly Thr Ser Arg Pro Val Gln Glu Gln	
595 600 605	
GGT GGG GCG CAG ACT TCA CTC CAT ACC CAG GGG TCC GGA CAA TGT GCA	1872
Gly Gly Ala Gln Thr Ser Leu His Thr Gln Gly Ser Gly Gln Cys Ala	
610 615 620	
GAA TGA	1878
Glu	
625	

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 625 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Met	Ala	Pro	Arg	Ala	Arg	Arg	Arg	Arg	Gln	Leu	Pro	Ala	Pro	Leu	Leu	1	5	10	15
Ala	Leu	Cys	Val	Leu	Leu	Val	Pro	Leu	Gln	Val	Thr	Leu	Gln	Val	Thr	20	25	30	
Pro	Pro	Cys	Thr	Gln	Glu	Arg	His	Tyr	Glu	His	Leu	Gly	Arg	Cys	Cys	35	40	45	
Ser	Arg	Cys	Glu	Pro	Gly	Lys	Tyr	Leu	Ser	Ser	Lys	Cys	Thr	Pro	Thr	50	55	60	
Ser	Asp	Ser	Val	Cys	Leu	Pro	Cys	Gly	Pro	Asp	Glu	Tyr	Leu	Asp	Thr	65	70	75	80
Trp	Asn	Glu	Glu	Asp	Lys	Cys	Leu	Leu	His	Lys	Val	Cys	Asp	Ala	Gly	85	90	95	
Lys	Ala	Leu	Val	Ala	Val	Asp	Pro	Gly	Asn	His	Thr	Ala	Pro	Arg	Arg	100	105	110	
Cys	Ala	Cys	Thr	Ala	Gly	Tyr	His	Trp	Asn	Ser	Asp	Cys	Glu	Cys	Cys	115	120	125	
Arg	Arg	Asn	Thr	Glu	Cys	Ala	Pro	Gly	Phe	Gly	Ala	Gln	His	Pro	Leu	130	135	140	
Gln	Leu	Asn	Lys	Asp	Thr	Val	Cys	Thr	Pro	Cys	Leu	Leu	Gly	Phe	Phe	145	150	155	160
Ser	Asp	Val	Phe	Ser	Ser	Thr	Asp	Lys	Cys	Lys	Pro	Trp	Thr	Asn	Cys	165	170	175	
Thr	Leu	Leu	Gly	Lys	Leu	Glu	Ala	His	Gln	Gly	Thr	Thr	Glu	Ser	Asp	180	185	190	
Val	Val	Cys	Ser	Ser	Ser	Met	Thr	Leu	Arg	Arg	Pro	Pro	Lys	Glu	Ala	195	200	205	
Gln	Ala	Tyr	Leu	Pro	Ser	Leu	Ile	Val	Leu	Leu	Leu	Phe	Ile	Ser	Val	210	215	220	
Val	Val	Val	Ala	Ala	Ile	Ile	Phe	Gly	Val	Tyr	Tyr	Arg	Lys	Gly	Gly	225	230	235	240
Lys	Ala	Leu	Thr	Ala	Asn	Leu	Trp	Asn	Trp	Val	Asn	Asp	Ala	Cys	Ser	245	250	255	
Ser	Leu	Ser	Gly	Asn	Lys	Glu	Ser	Ser	Gly	Asp	Arg	Cys	Ala	Gly	Ser	260	265	270	
His	Ser	Ala	Thr	Ser	Ser	Gln	Gln	Glu	Val	Cys	Glu	Gly	Ile	Leu	Leu	275	280	285	
Met	Thr	Arg	Glu	Glu	Lys	Met	Val	Pro	Glu	Asp	Gly	Ala	Gly	Val	Cys	290	295	300	

Gly Pro Val Cys Ala Ala Gly Gly Pro Trp Ala Glu Val Arg Asp Ser
 305 310 315 320
 Arg Thr Phe Thr Leu Val Ser Glu Val Glu Thr Gln Gly Asp Leu Ser
 325 330 335
 Arg Lys Ile Pro Thr Glu Asp Glu Tyr Thr Asp Arg Pro Ser Gln Pro
 340 345 350
 Ser Thr Gly Ser Leu Leu Leu Ile Gln Gln Gly Ser Lys Ser Ile Pro
 355 360 365
 Pro Phe Gln Glu Pro Leu Glu Val Gly Glu Asn Asp Ser Leu Ser Gln
 370 375 380
 Cys Phe Thr Gly Thr Glu Ser Thr Val Asp Ser Glu Gly Cys Asp Phe
 385 390 395 400
 Thr Glu Pro Pro Ser Arg Thr Asp Ser Met Pro Val Ser Pro Glu Lys
 405 410 415
 His Leu Thr Lys Glu Ile Glu Gly Asp Ser Cys Leu Pro Trp Val Val
 420 425 430
 Ser Ser Asn Ser Thr Asp Gly Tyr Thr Gly Ser Gly Asn Thr Pro Gly
 435 440 445
 Glu Asp His Glu Pro Phe Pro Gly Ser Leu Lys Cys Gly Pro Leu Pro
 450 455 460
 Gln Cys Ala Tyr Ser Met Gly Phe Pro Ser Glu Ala Ala Ala Ser Met
 465 470 475 480
 Ala Glu Ala Gly Val Arg Pro Gln Asp Arg Ala Asp Glu Arg Gly Ala
 485 490 495
 Ser Gly Ser Gly Ser Ser Pro Ser Asp Gln Pro Pro Ala Ser Gly Asn
 500 505 510
 Val Thr Gly Asn Ser Asn Ser Thr Phe Ile Ser Ser Gly Gln Val Met
 515 520 525
 Asn Phe Lys Gly Asp Ile Ile Val Val Tyr Val Ser Gln Thr Ser Gln
 530 535 540
 Glu Gly Pro Gly Ser Ala Glu Pro Glu Ser Glu Pro Val Gly Arg Pro
 545 550 555 560
 Val Gln Glu Glu Thr Leu Ala His Arg Asp Ser Phe Ala Gly Thr Ala
 565 570 575
 Pro Arg Phe Pro Asp Val Cys Ala Thr Gly Ala Gly Leu Gln Glu Gln
 580 585 590
 Gly Ala Pro Arg Gln Lys Asp Gly Thr Ser Arg Pro Val Gln Glu Gln
 595 600 605
 Gly Gly Ala Gln Thr Ser Leu His Thr Gln Gly Ser Gly Gln Cys Ala
 610 615 620

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Arg	Met	Lys	Gln	Ile	Glu	Asp	Lys	Ile	Glu	Glu	Ile	Leu	Ser	Lys	Ile
1				5				10						15	
Tyr	His	Ile	Glu	Asn	Glu	Ile	Ala	Arg	Ile	Lys	Lys	Leu	Ile	Gly	Glu
			20					25					30		

Arg

CLAIMS

We claim:

1. An isolated DNA selected from the group consisting of:

5 (a) a DNA encoding a protein having an amino acid sequence as set forth in SEQ ID NO:6, wherein the protein has an amino terminus selected from the group consisting of an amino acid between amino acid 1 and amino acid 33, inclusive, of SEQ ID NO:6, and a carboxy terminus selected from the group consisting an amino acid between amino acid 196 and amino acid 616, inclusive;

10 (b) a DNA encoding a protein having an amino acid sequence as set forth in SEQ ID NO:15, wherein the protein has an amino terminus selected from the group consisting of an amino acid between amino acid 1 and amino acid 31, inclusive, of SEQ ID NO:15, and a carboxy terminus selected from the group consisting an amino acid between amino acid 197 and amino acid 625, inclusive;

15 (c) DNA molecules capable of hybridization to the DNA of (a) or (b) under stringent conditions, and which encode biologically active RANK; and

(d) DNA molecules encoding fragments of proteins encoded by the DNA of (a), (b) or (c).

20 2. The isolated DNA of claim 1, which encodes a RANK polypeptide that is at least about 80% identical in amino acid sequence to the native form of RANK

3. The isolated DNA of claim 1, which encodes a soluble RANK polypeptide.

25 4. The isolated DNA of claim 2, which encodes a soluble RANK polypeptide.

30 5. The isolated DNA of claim 3, which further comprises a DNA encoding a polypeptide selected from the group consisting of an immunoglobulin Fc domain, an immunoglobulin Fc mutein, a FLAGTM tag, a peptide comprising at least about 6 His residues, a leucine zipper, and combinations thereof.

35 6. The isolated DNA of claim 4, which further comprises a DNA encoding a polypeptide selected from the group consisting of an immunoglobulin Fc domain, an immunoglobulin Fc mutein, a FLAGTM tag, a peptide comprising at least about 6 His residues, a leucine zipper, and combinations thereof.

7. A recombinant expression vector comprising a DNA sequence according to claim 1.

8. A recombinant expression vector comprising a DNA sequence according to claim 2.

9. A recombinant expression vector comprising a DNA sequence according to claim

3.

10. A recombinant expression vector comprising a DNA sequence according to claim

4.

11. A recombinant expression vector comprising a DNA sequence according to claim

5.

12. A recombinant expression vector comprising a DNA sequence according to claim

6.

13. A host cell transformed or transfected with an expression vector according to claim 7.

14. A host cell transformed or transfected with an expression vector according to claim 8.

15. A host cell transformed or transfected with an expression vector according to claim 9.

16. A host cell transformed or transfected with an expression vector according to claim 10.

17. A host cell transformed or transfected with an expression vector according to claim 11.

18. A host cell transformed or transfected with an expression vector according to claim 12.

19. A process for preparing a RANK protein, comprising culturing a host cell according to claim 13 under conditions promoting expression and recovering the RANK .

20. A process for preparing a RANK protein, comprising culturing a host cell according to claim 14 under conditions promoting expression and recovering the RANK .

21. A process for preparing a RANK protein, comprising culturing a host cell according to claim 15 under conditions promoting expression and recovering the RANK .

5 22. A process for preparing a RANK protein, comprising culturing a host cell according to claim 16 under conditions promoting expression and recovering the RANK .

23. A process for preparing a RANK protein, comprising culturing a host cell according to claim 17 under conditions promoting expression and recovering the RANK .

10 24. A process for preparing a RANK protein, comprising culturing a host cell according to claim 18 under conditions promoting expression and recovering the RANK .

15 25. An isolated DNA selected from the group consisting of oligonucleotides of at least about 17 nucleotides in length, oligonucleotides of at least about 25 nucleotides in length, and oligonucleotides of at least about 30 nucleotides in length, which is a fragment of the DNA of SEQ ID NO:5 or SEQ ID NO:14.

26. An isolated RANK polypeptide selected from the group consisting of:

20 (a) a polypeptide having an amino acid sequence of amino acids 33 through 196 of SEQ ID NO: 6;

(b) a polypeptide having an amino acid sequence of amino acids 31 through 197 of SEQ ID NO: 15;

25 (c) a RANK polypeptide encoded by a DNA capable of hybridization to a DNA encoding the protein of (a) or (b) under stringent conditions, and which is biologically active; and

(d) fragments of the polypeptides of (a), (b) or (c) which are biologically active.

30 27. The protein according to claim 26, having an amino acid sequence at least about 80% identical to SEQ ID NO:6 or SEQ ID NO:15.

28. The protein according to claim 27, which is a soluble RANK.

29. The protein according to claim 26, which is a soluble RANK.

35 30. A soluble RANK protein which further comprises a peptide selected from the group consisting of an immunoglobulin Fc domain, an immunoglobulin Fc mutein, a FLAG™ tag, a peptide comprising at least about 6 His residues, a leucine zipper, and combinations thereof.

31. An antibody immunoreactive with RANK polypeptide according to claim 26.

32. The antibody according to claim 31, which is a monoclonal antibody.

5

33. A method of inhibiting activation of NF κ B, comprising contacting a cell that expresses membrane-associated RANK with a soluble RANK and allowing the soluble RANK to bind RANKL and inhibit binding thereof to the cell.

10

34. A method of regulating an immune or inflammatory response, comprising administering a soluble RANK polypeptide composition to an individual at risk for an immune or inflammatory response, and allowing the soluble RANK to bind RANKL and inhibit binding thereof to cells expressing RANK.

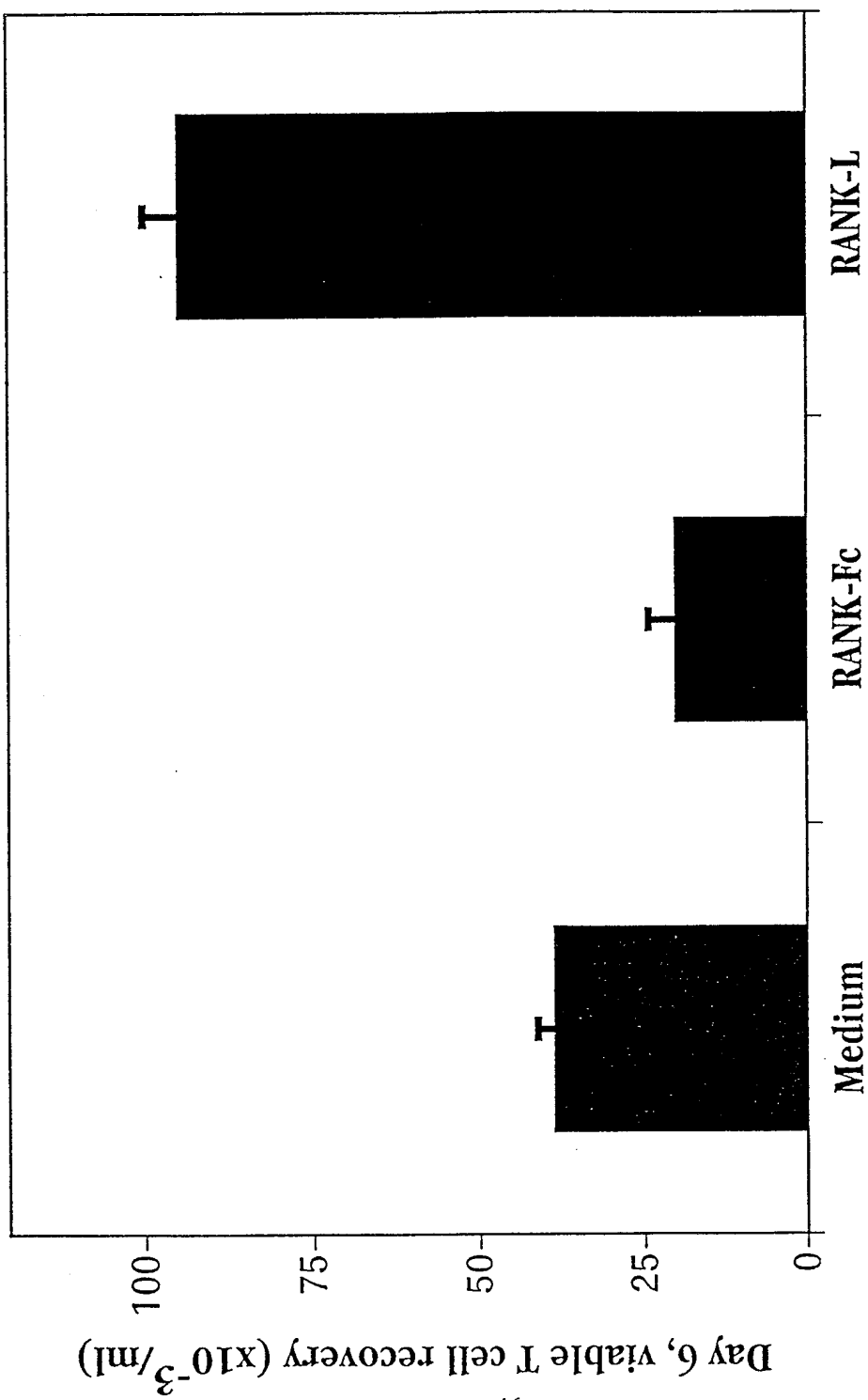


Figure 1

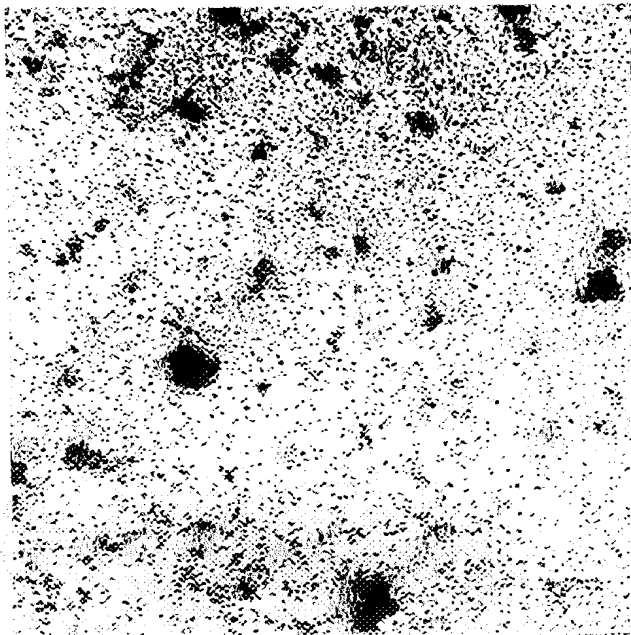


Fig. 2A

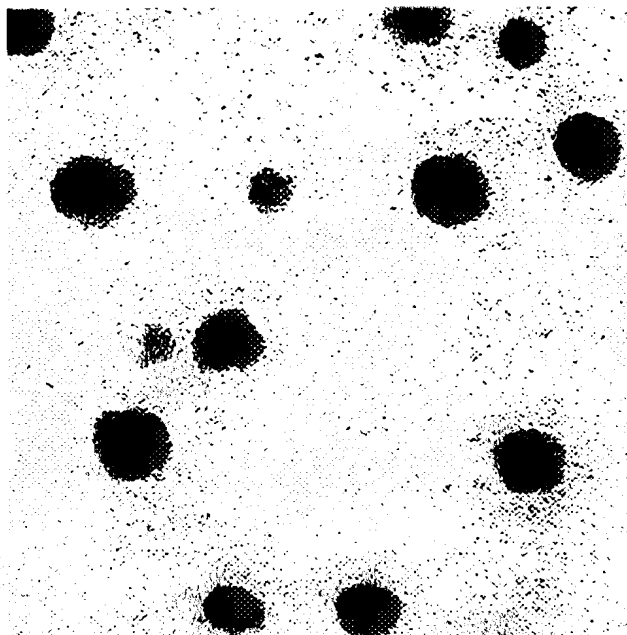


Fig. 2B

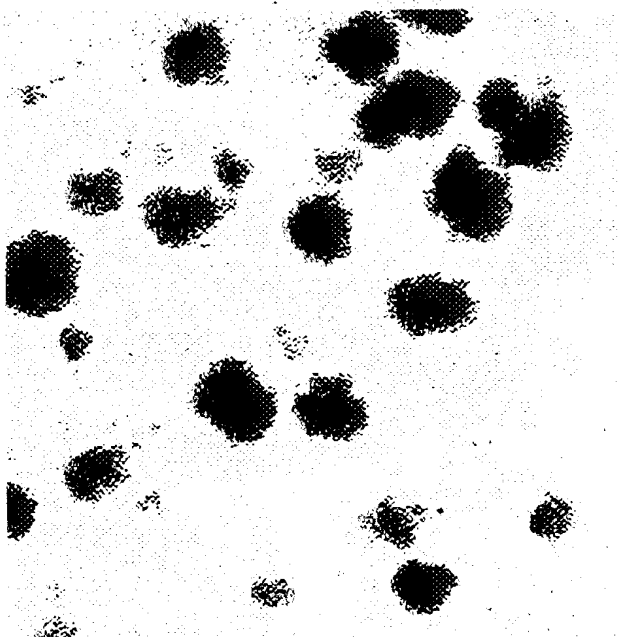


Fig. 2C

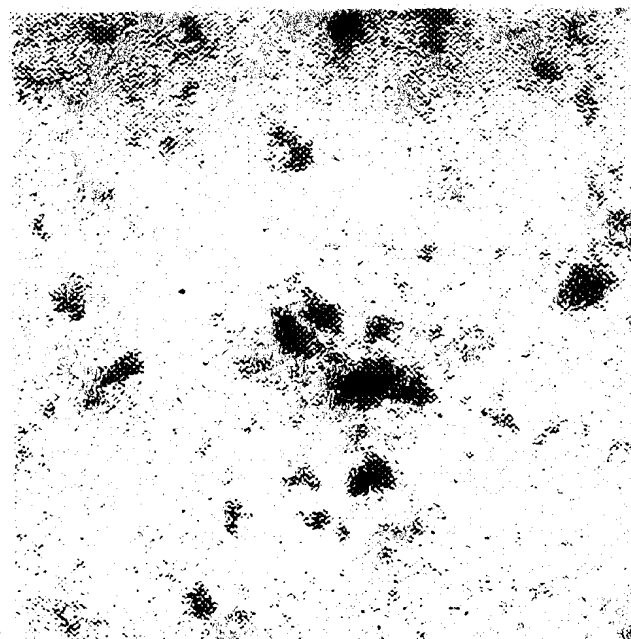


Fig. 2D

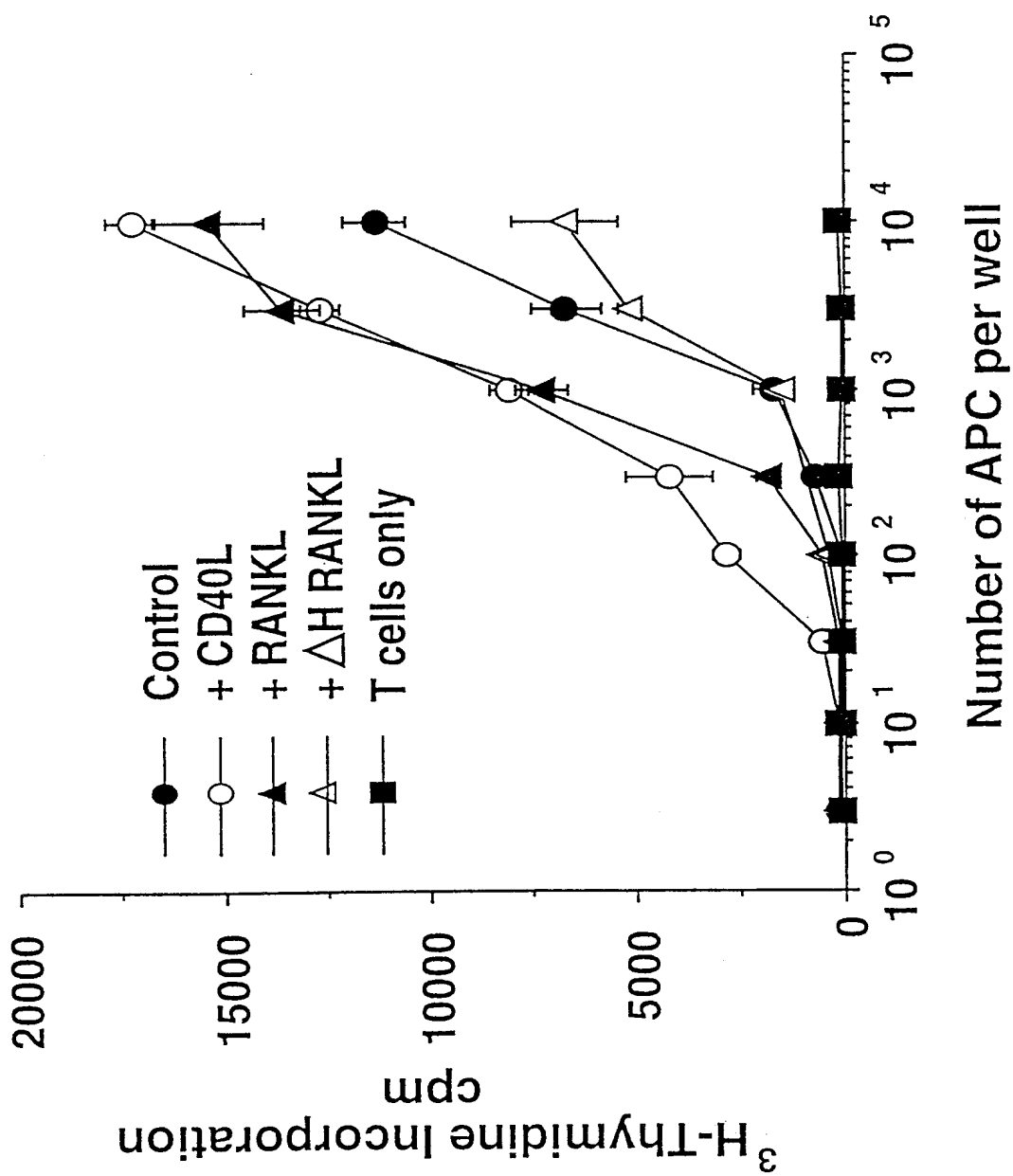
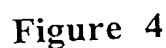


Figure 3



[illegible]

Figure 5

Htnfa	..	STHVL	LA	T	HTISR	IAVSY	QTKV	PNL	LSSA	I	KSPC	QRET	PE	GAEAK
Htnfb	KA	TSSP	LL	LA	HEVQ	LFNSKY	PFHD	VL	LSSQ	KMSA	KMVY	CTTG	QAE	PGLQE
Hfasl	ENTK	NDKQ	LV	M	HKVY	KYTS	PDPI	LL	MMKSA	RNS	STK	YCW	SGN	WA
Htrankl	DLAT	EYHPT	T	T	QYIY	KTS	PS	SH	MMKGG	STK	YCW	SGN	SE	YF
Hcd271	TAS	RRHPT	T	T	LVG	ICSPAS	RS	IS	LLRRL	TH	SH	QGC	TI	..
Hcd401	REAS	SSQA	PI	I	ASL	CLKSPGR	FE	RI	LLRAAN	PAS	SE	ARNSA	FG	..
H41bb1	GS	GS	VS	LA	LH	PLR	SA	AG	LV	DL
Hcd301
Htnfa	PWY	EP	IY	LG	G	LEK	GD	R	Y	PH	LDFA	..	ES	GG
Htnfb	PWL	H	SM	YH	GA	LTQ	GD	Q	Y	PH	LVLS	..	PS	T
Hfasl
Htrankl
Hcd271
Hcd401
H41bb1
Hcd301
Htnfa	IA	L
Htnfb	AF	AL
Hfasl	LY	KL
Htrankl	AF	LV
Hcd271	AF	KV	RD	ID
Hcd401	VQ	WV	RP
H41bb1	LL	KL
Hcd301	LF	RV	TP	EI	PA

Figure 5 (cont.)

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12N 15/12, 15/11, 15/62, C07K 14/715, 16/28		A3	(11) International Publication Number: WO 98/28424
			(43) International Publication Date: 2 July 1998 (02.07.98)
(21) International Application Number: PCT/US97/23866 (22) International Filing Date: 22 December 1997 (22.12.97) (30) Priority Data: 60/059,978 23 December 1996 (23.12.96) US 08/813,509 7 March 1997 (07.03.97) US 60/064,671 14 October 1997 (14.10.97) US (71) Applicant: IMMUNEX CORPORATION [US/US]; Law Dept., 51 University Street, Seattle, WA 98101 (US). (72) Inventors: ANDERSON, Dirk, M.; 3616 N.W. 64th Street, Seattle, WA 98107 (US). GALIBERT, Laurent, J.; 617 5th Avenue West, Seattle, WA 98119 (US). MARASKOVSKY, Eugene; 4123 Evanston Avenue North, Seattle, WA 98103 (US). (74) Agent: PERKINS, Patricia, Anne; Immunex Corporation, Law Dept., 51 University Street, Seattle, WA 98101 (US).			(81) Designated States: AL, AM, AU, BA, BB, BG, BR, CA, CN, CU, CZ, DK, EE, GE, HU, IL, IS, JP, KP, KR, LC, LK, LR, LT, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, SL, TR, TT, UA, UZ, VN, YU, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> (88) Date of publication of the international search report: 11 September 1998 (11.09.98)
(54) Title: RECEPTOR ACTIVATOR OF NF-KAPPA B, RECEPTOR IS MEMBER OF TNF RECEPTOR SUPERFAMILY			
(57) Abstract Isolated receptors, DNAs encoding such receptors, and pharmaceutical compositions made therefrom, are disclosed. The isolated receptors can be used to regulate an immune response. The receptors are also useful in screening for inhibitors thereof.			

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/23866

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/12 C07K14/715 C12N15/11 C07K16/28 C12N15/62

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	ANDERSON DM ET AL: "A homologue of the TNF receptor and its ligand enhance T-cell growth and dendritic-cell function." NATURE, NOV 13 1997, 390 (6656) P175-9, ENGLAND, XP002065548 see figure 1	1-34
A	--- WO 93 08207 A (IMMUNEX CORP) 29 April 1993 see SEQ ID NO 4	3,29
X	--- EMBL Databasse entry HS421358 Accession number W74421, 21 Jun 1996 HIILIER L ET AL: Homo sapiens cDNA clone 346544 contains Alu repetitive element XP002065589 see sequence -----	25

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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Date of the actual completion of the international search

22 May 1998

Date of mailing of the international search report

07.07.98

Name and mailing address of the ISA

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Fax: (+31-70) 340-3016

Authorized officer

Espen, J

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 97/23866

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 33,34 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 97/23866

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9308207 A	29-04-1993	AU 661360 B	20-07-1995
		AU 3122693 A	21-05-1993
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		EP 0667901 A	23-08-1995
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		JP 7504083 T	11-05-1995
		NO 941422 A	27-06-1994
		NO 980030 A	05-01-1998
		US 5716805 A	10-02-1998
